

nature*August 19, 1976*

Shadowy threshold

THE ridiculous consequences of the threshold nuclear test-ban treaty, pressed on an unwilling world in 1974 and scheduled to take effect in April 1976, begin to make themselves known. The treaty is a strictly bilateral affair between the United States and the Soviet Union and the two parties have agreed to restrain weapons to yields not exceeding 150 kilotons. A related treaty covered peaceful nuclear explosions. Although formal ratification is still awaited, both parties have been honouring the treaty for some months.

In 1974 there was vigorous criticism of almost all aspects of the treaty in many parts of the world: a bilateral treaty was too coy; the threshold was too high for any sensible arms control purposes; the complications of geology made accurate seismic monitoring of the threshold difficult. On the last count, the period of nearly two years between proposal and enforcement was put forward by the treaty's proponents as a time when data exchange would allow accurate calibrations of test sites to be made. Certainly there was a mild flurry of activity as scientists moved between capitals clutching seismograms. But on July 4, in the case of one of the first tests to be fired by the Soviet Union since the April deadline, there was a sufficient element of doubt in American official quarters to push the whole concept of the treaty right back into the public arena. The arguments have since continued.

No official American estimate of the yield has been forthcoming but the figures first being passed around were 100 to 200 kilotons; a later figure to be mentioned was "exactly 150 kilotons". In the midst of all this an unpublished understanding between the two powers was wheeled out, to the effect that "technical uncertainties . . . may result in slight unintended breaches . . . one or two such per year would not be considered a violation of the treaty". In other words, since yields can be pre-determined to within a few per cent knowing the conformation of the device, at least one party to the treaty—

probably the Soviet Union—intended to test right up to the threshold. Some arms control measure.

There has been much justifiable tut-tutting over these loose edges to the treaty in the past week, but little about the calculations which have helped provoke it. Yet there is a vast amount of seismic data in the public domain and this explosion will have been excellently recorded all over the world. United States government estimates take into account we know not what, but it is open to anyone else to make his or her own reasonable estimate. There are published papers giving clear recipes for how to convert a seismic signal to explosive yield, with the help of some modest knowledge of Soviet geology. And when we do the sums we can't see how the yield can be much more than 100 kilotons.

The cause of the discrepancy is an old problem. It is easiest to measure the yield seismically by observation of the so-called body waves generated by the explosion. These have the larger signal-to-noise ratio and are measured routinely. The so-called surface waves from blasts, on the other hand, are smaller and need a little more time for their analysis. But at the sort of yields we are talking about there begin to be discrepancies between yields from the same explosion measured routinely by the two techniques—and there are good theoretical grounds for favouring the surface-wave method.

In the past, the body-wave method has been known seriously to overestimate the yield. This has led to the publication of information about the yields of Soviet tests, placing them in the multi-megaton region when the yield has probably been barely a megaton. Admittedly all estimates of seismic yield are accurate only to perhaps 20%. But now we see an old problem emerging to befuddle the issue of the threshold treaty, which is made to look silly right from the word go. One wonders whether the two years could not have been more profitably spent in avoiding such a hassle. □



Toxic cloud over Seveso

The fall-out from last month's explosion in Italy involves more than dust, as **Alastair Hay** points out

ON JULY 10, an explosion at a chemical factory in the North Italian town of Seveso released a cloud of vapour which contaminated the surrounding area. The vapour, a chemical cocktail consisting primarily of trichlorophenol (TCP), also contained an extremely toxic by-product, TCDD (2,3,7,8 - tetrachlorodibenzopara - dioxin), a virulent poison.

It happened six and a half hours after the plant, belonging to Givaudan ICMESA, a subsidiary of Hoffman-La Roche, had closed down for the weekend. There was a "runaway reaction" in the reactor producing trichlorophenol. The pressure increased as the reaction became exothermic, and reached the critical limit, blowing the safety valve and thus discharging the reactor contents. The valve, situated outside the plant, discharged the hot vapour directly into the atmosphere. When the solvents evaporated, trichlorophenol crystals and 2 kg of TCDD, in the form of dust, were precipitated south of the factory over an area inhabited by some two thousand people.

Local police were informed of the explosion on the day that it occurred; the following day the mayor of Seveso was told; and on Monday, July 12, ICMESA notified the local authorities of areas bordering on Seveso. A statement was issued by these local officials, warning against the consumption of fruit and other crops from the area surrounding the factory. On July 15, the first animal deaths were reported. The first reports of children suffering from skin rashes came a day later. By July 22, when over thirty people were reported to be showing symptoms of burns and poisoning, local authorities were still insisting that there was no cause for panic. No general evacuation had been planned.

Not until two weeks after the explosion was TCDD first identified, by a provincial diagnostic centre. The identity of the dioxin was confirmed by Givaudan three days later, on July 26. Local officials had meanwhile been alerted to the seriousness of the situation by the ICMESA management, and on Tuesday, July 27, fully 2½ weeks after the explosion, evacuation began, at first only of children. Since then the known area of serious contamination has grown severalfold, and by the middle of last week over 800 people had been evacuated from this zone. Another 1,300 children had been

evacuated, as a precautionary measure, from the area outside that of immediate contamination.

Delayed action

Only after it had been established that TCDD had also been released in the vapour cloud did the authorities realise the need for urgent action. Up to this point, the information supplied to them by the ICMESA management had implied that the worst component of the vapour was trichlorophenol. This undoubtedly led the local authorities to believe that the situation, although serious, did not warrant a general exodus from the contaminated area.

It is this delay which has given cause for concern, particularly in view of the known toxicity of the dioxins. Experimental evidence is available of the effects of TCDD on a range of animals, and is to be found in the US National Academy of Sciences (NAS) document *Effects of Herbicides in South Vietnam*. A single dose of this dioxin is lethal in guinea pigs at a concentration of $0.6 \mu\text{g kg}^{-1}$. In male rats the lethal dose is $22 \mu\text{g kg}^{-1}$; in rabbits $115 \mu\text{g kg}^{-1}$; and in humans, it has been calculated to be less than 5 mg kg^{-1} . This means that the toxicity of TCDD can be compared to that of strychnine.

Clinical signs of TCDD poisoning include atrophy of the kidney; necrotic changes of the liver; ulceration of the stomach; haemorrhages in the gastrointestinal tract and other organs; and atrophy of the thymus and other lymphoid organs and tissues. The latter changes are those most commonly observed.

Teratogenicity of TCDD has been demonstrated in mice, although evidence of teratogenic effects in other animals is more equivocal. According to Neubert *et al.* (*Environ. Health Perspectives*, Exp. Issue No. 5, 67-80; 1973), when TCDD was given to mice daily on days 6 to 15 of pregnancy, at least 50% of the progeny exhibited kidney anomalies at concentrations of $1-3 \mu\text{g kg}^{-1}$; death occurred at concentrations of $7 \mu\text{g kg}^{-1}$.

Reports from Seveso confirm that 500 people are being treated for poisoning. Many are showing symptoms of kidney and liver malfunction. Others from the area affected by TCDD fall-out have chloracne, a persistent and disfiguring form of acne associated with contact with chlorinated compounds. If treated, the acne may clear up in six months. There is evidence,

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however, of a worker in a chemical factory producing chlorophenols who is still disfigured 15 years after exposure to the chemicals.

Not the first time

As the painful assessment begins of the effects of the dioxin contamination, evidence has accrued almost daily of chemical explosions involving trichlorophenol production which have already occurred and which were similar to the one at the ICMESA plant.

The first accident occurred in 1953 at the Ludwigshafen works of the Badische Anilin und Soda Fabrik AG (BASF) in Germany. Fifty-five workers were exposed to dioxin, all of whom developed chloracne. Twenty-one developed symptoms of systemic poisoning, including damage to the liver, kidney and spleen; the heart, respiratory tract, eyes and nervous system were also affected. Five years after the accident, a worker engaged on repair work on the site developed symptoms of dioxin poisoning; nine months after the appearance of the first symptoms, this worker died from an inflammation of the pancreas. Following medical advice, BASF later stopped production of trichlorophenol.

In 1963, at the Philips Duphar complex in Amsterdam, an explosion released between 30 and 200 g of dioxin into the factory hall. Fifty people were affected, at least ten of whom are still suffering from skin complaints. Four workers died within two years of the accident, but there is no proven connection between their deaths and dioxin poisoning. After an unsuccessful attempt to remove dioxin from the factory walls, the firm closed down the trichlorophenol plant for ten years. When information on dioxin contamination had been reviewed, the only course of action open to the company was to dismantle the plant brick by brick working from the inside outwards. The rubble, embedded in con-

crete, was eventually dumped into the Atlantic.

At the Coalite and Chemical Products plant at Bolsover, Derbyshire (UK), seventy-nine men developed chloracne following an explosion at the trichlorophenol plant in 1968. Since the Seveso accident, this firm and the West German firm of Bayer of Leverkusen have both temporarily ceased production and are reviewing their manufacturing procedures.

The most disturbing reports relating to dioxin poisoning, however, are undoubtedly those from Vietnam. Increasing criticism of the American use of herbicides in the war in Indochina resulted in the National Academy of Sciences conducting a thorough investigation. The report of their findings, published in 1974, has been criticised over its interpretation of the data concerning deaths and birth defects in communities sprayed with herbicides. In an introduction to the report, Dr Philip Handler, the NAS President, claimed that no definite connection existed to confirm findings of herbicide-induced birth defects or deaths.

The NAS report itself does, however, contain considerable information about the effects of herbicides and the contaminant TCDD on humans, animals and vegetation in Vietnam. One anthropologist who submitted evidence to the NAS study, Dr Gerald Hickey of the South-East Asia Programme of Cornell University, has reported evidence of deaths from among the children of the Montagnard peoples of South Vietnam, deaths directly related to herbicide spraying. The first-hand interviews he conducted with Montagnard villagers revealed the additional evidence that other children had shown symptoms such as skin rashes, diarrhoea and abdominal pains.

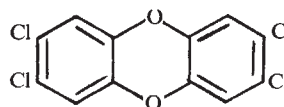
Hickey was cautious in attributing these effects directly to TCDD; the evidence is more circumstantial. During the Vietnam war, the Americans repeatedly sprayed these mountainous areas of South Vietnam, inhabited by the Montagnard people, with the herbicide "Agent Orange", a 50:50 mixture of the n-butyl esters of 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid). The dioxin TCDD is a contaminant present in 2,4,5-T at levels variously estimated at between 0.07 and 50 p.p.m.

Although the NAS report claimed that no evidence is available to substantiate herbicide-induced birth defects in humans, the Italian authorities are currently paying serious attention to the advice of a Vietnamese doctor. Professor Ton That Tung of the Viet Duc Hospital in Hanoi insists (in *Vietnamese Studies*, 29, 53-81; 1971) that such evidence is available.

Unanswered questions

Certainly the assembled evidence points to some unanswered questions. Because previously recorded accidents at other trichlorophenol-producing plants had released TCDD, the chance existed that the same would be true in any accident at Seveso, and the authorities might have expected to have been informed accordingly. But even in the unlikely event that the ICMESA management was unaware of the historical

evidence pointing to this danger, simple theoretical knowledge of the factors favouring TCDD production should have alerted them to the possibility of dioxin contamination. Moreover, the plant's safety valve was situated outside the plant, allowing a discharge of vapour directly into the atmosphere. This served to protect the plant itself from the vapour, but did so at the cost of serious contamination of the surrounding countryside.



TCDD

(2,3,7,8-tetrachlorodibenzopara-dioxin)

TCDD is produced as a by-product during the alkaline hydrolysis of tetrachlorobenzene with methanolic sodium hydroxide to form 2,4,5-trichlorophenol (1).

Trichlorophenol is both the industrial precursor of 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), a herbicide normally used in esterified form as the butyl ester (2), and an intermediate product in the manufacture of hexachlorophene, an antibacterial agent added to soaps, shampoos, deodorants and toothpastes.

The hydrolysis of tetrachlorobenzene is carried out at high temperatures and under pressure. This reaction, if not very carefully controlled, is favourable to the formation of TCDD, the most common chlorodibenzoparadioxin present in 2,4,5-T. Other toxic polychlorodibenzoparadioxins are also produced in the manufacture of chlorinated phenols.

Theoretically, at least 75 different chlorodibenzoparadioxins can be predicted having one to eight chlorine atoms attached to the two benzene rings. At least ten have been synthesised, and one or two more have been found in food, animal feeds and 2,4,5-T.

Hexachlorophene is prepared by the

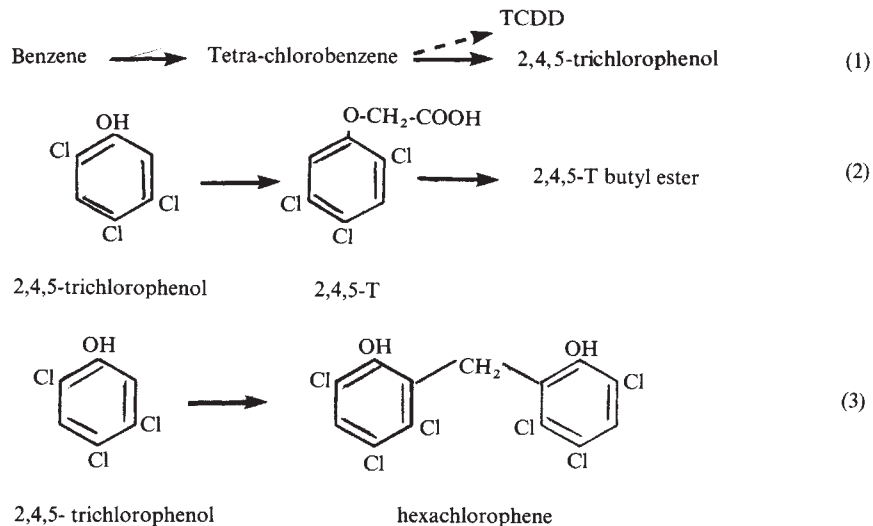
condensation of two molecules of 2,4,5-trichlorophenol with formaldehyde in the presence of concentrated sulphuric acid (3). The preparation procedure has been improved, and the Givaudan Corporation hold the US patents Nos. 2,435,593 (1948) and 2,812,365 (1957).

Properties of TCDD

Like other chlorodioxins, TCDD is stable to heat, acids and alkali; a temperature of 800 °C is required for thermal decomposition. TCDD is virtually insoluble in water (0.0002 p.p.m.), only slightly soluble in fats (44 p.p.m. in lard oil), more soluble in hydrocarbons (570 p.p.m. in benzene), and at its most soluble in chlorinated organic solvents (1400 p.p.m. in ortho-dichlorobenzene).

Uptake of TCDD occurs slowly in plants (oats and soybeans). No accumulation above external concentration occurs, however, and plants grow to maturity (A. R. Isensee and G. E. Jones, *Agric. Food Chem.*, 19, 1210-1214; 1971).

TCDD is almost immobile in soil. Even in very porous soil it remains close to the point of application (P. C. Kearney *et al.*, *Environ. Health Perspectives*, Exp. Issue No. 5, 273-278; 1973).



The question of decontamination is now presenting Givaudan, Roche and the Italian government with serious problems. An Italian government commission recommended removal of topsoil to a depth of 10 cm in an area of 280 acres, the dismantling of all buildings in the Seveso area and the total disruption of all wildlife. These recommendations may take up to three years to implement. The sheer volume of soil to be processed and the number of buildings involved will create enormous technical problems of disposal. Great care will have to be taken to avoid further contamination by TCDD when material is removed from Seveso.

The British firm of Cremer and Warner have been retained by Givaudan to help in the task of decontamination. Cremer and Warner are chemical engineers specialising in the environmental safety aspects of industrial pollution. Data already accumulated indicate that the intensity of contamination does not conform to any uniform pattern, and the feeling is that it would be premature to implement the Italian commission's proposals, at least until potential alternatives are considered.

But no one doubts that a satisfactory policy for accident prevention and control can possibly be formulated if vital information is withheld from

those whom it may affect most directly. In Seveso, the local mayors did make attempts to find out what was actually being produced at the ICMESA plant. But that was four years ago. Only last year did Givaudan finally provide some information. No mention was made of the dioxin risk, and therefore no safety measures were suggested. Even now there is conflicting information about the processes for which the ICMESA works were designed. One senior Roche executive has said the plant was producing trichlorophenol for the manufacture of the herbicide 2,4,5-T; another spokesman claimed that the plant was making trichlorophenol for the manufacture of hexachlorophene at another site.

Few unaffected

Few people have been uninvolved by the actions of the Roche subsidiary. Scientists and engineers at the Seveso plant failed to predict the extent of dioxin contamination and its spread over the countryside. Workers at the factory demanded to know of any dangers involved. Management did not inform them until two weeks after the explosion. Local residents knew nothing of the real hazards of the contamination. The medical profession, lacking an antidote, must give only symptomatic treatment. Pregnant

women, agonising over the teratogenic effects of TCDD, must decide with little firm guidance from the Church whether to undergo abortions. Ecologists cannot predict the long term effects of dioxin contamination. The authorities know that the decontamination proposals themselves are at best a mixture of policies adopted at previous accidents, leavened with a good deal of hope.

The suggestions being made now involve at the very least the implementation of strict codes of practice, to be adopted between industrial concerns and the local authorities to which they bear a great measure of responsibility. Such codes would include detailed procedures to be followed in the event of an accident. As for the withholding of information, few are accepting the obvious convenience this represents for manufacturers as a satisfactory explanation. Many are saying that with all the facts more freely available, it may, for example, be decided that the risks involved in producing trichlorophenol outweigh the necessity of a 2,4,5-T weedkiller—or a TCP mouthwash. Only the inquest now to come will reveal whether some sort of political solution is needed to ensure that the necessary information is available and to prevent a recurrence of this kind of disaster. □

Europe's ageing research staffs

Mike Duckenfield reports from Stockholm on the problem of an "unhealthy" age profile among researchers and teachers

WITH one or two exceptions, notably Finland where spending is to be increased from 1% to 1.7% of the GNP during the next five years, research budgets in most Western European countries are having a struggle to keep pace with rising costs. Not only is government spending being cut back or slowed down, but what funds there are available are being affected by an incremental drift of salaries as young staff get older and, as in Britain and the USA, increasing unionisation. Even in Norway, where unemployment is well below 2% and economic growth at a steady 7%, the prospects for those wanting research jobs are very slim.

Graduate unemployment is a problem in itself, but there is also another side to the coin: the likely prospect of universities and research institutes in most nations losing a generation of teachers and researchers during the next 15 years due to the lack of job opportunities. The problem, in short, is that the universities are now moving

into the shadow of the massive expansion of higher education which took place in the 1960s. Due to this boom the average age of teachers and researchers is now very low, retirements are relatively few and future openings for new blood and ideas drastically diminished. According to academics in several countries, promotion, mobility and possibly even creativity are all likely to suffer.

The problem of an "unhealthy" age profile among researchers and teachers is beginning to be studied in nations ranging from France to Norway. Due to large scale recruitment in the middle and late 1960s, too large a proportion of staff are now only at the beginning of their careers. A recent Federal German survey shows that while 45.9% of professors and senior lecturers in 1966 were over 50 years old and only 22.3% under 40, corresponding figures for 1972 were 28% and 30.5%. In addition, while other teaching staff under 35 accounted for 17.4% of the total in 1966, six years later the figure

was 27.3%.

In Britain, too, the average age of university teachers has fallen significantly; now 63% of academic staff are under 40, 26% are under 30 and 13% over 50. In Norway 18.8% of staff are over 50 compared with 43.7% under 35, while a survey of French research workers in the Centre National de la Recherche Scientifique showed the average age of those engaged in mathematics was 28 years and 7 months and those in physics and chemistry 34 years and 5 months. In Sweden as many as two-thirds of researchers in some branches of social science are under 35.

With comparatively young staff holding most of the senior positions, future promotion prospects are well below normal. A report prepared for the Council of Europe by Gert Elstermann, academic director of the University of the Saarlands, says that the annual replacement demand for teachers in Federal German universities and colleges up to 1990 will remain much the same as it is at present in law and the social sciences. For other subjects it will stay unchanged until the mid-1980s. Similarly, in Norway prospects for the next 15 years suggest annual recruitment levels below those of any year since the early 1960s. In

1972 there were 270 new jobs, this year only about 30 are expected.

Elstermann calculates that while an assistant lecturer in Germany had more than a 70% chance of being appointed as an established university teacher—in many cases as full professors—in the 1960s, in the 1976–85 period these chances will fall to only 15% before rising slowly to 23% in 1990 and 30% in the year 2000. Writing in last year's annual Norwegian research review, Hans Skoie, deputy director of Norway's Science and Humanities Research Council's Institute for Studies in Research and Higher Education, commented: "There is a definite danger that universities and research establishments may develop into communities of senior citizens leaving membership of future classes of graduates with miniscule chances of getting research jobs."

He fears that potential scientists will be discouraged from going on to higher studies and many of the best talents lost. This in turn would lead to less research being performed through

thesis work, thereby deteriorating the chances of senior staff to carry out their own projects. The trend against further studies has been evident for several years in neighbouring Sweden. In 1968, 37% of graduates went on to further studies. By 1971 this had fallen to 22.5% and last year it was down to only 14%. The Swedes are especially worried about their failure to attract students in mathematics and the natural sciences.

Another fear is that as the present research population gets collectively older fewer will be likely to change jobs—a trend reinforced by the tight employment market. This lack of mobility could then affect contacts between the universities, research institutes and commercial laboratories, and a Council of Europe report presented recently to a meeting of the organisation's committee for higher education and research concludes that the overall result could be stagnation and a possible decline in research creativity.

Attempts to overcome the age im-

balance problem vary widely. In France priority is being given to the recruitment of contractual staff as against creating new tenured positions which would have the status and security of those of civil servants. In Federal Germany the remedy advocated has been a strict adherence to temporary contracts, with nearly all staff below professorial level being untenured, the idea being to maintain a steady flow of young academics through the universities. However, long term solutions of this kind are almost certain to be opposed by the trade unions.

Other suggestions include encouraging older researchers to apply for senior administrative posts, introducing productivity or merit increments instead of those based on seniority or simply age, making the retirement age more flexible, offering increased opportunities for leave of absence, more academic exchange programmes and, perhaps rather optimistically, simply filling staff vacancies as soon as they occur. □

CHEMICAL WEAPONS

Disarmament by phases

At the Conference of the Committee on Disarmament (CCD) in Geneva, Britain last week tabled a draft convention on the prohibition and destruction of chemical weapons to complement the 1925 protocol banning their use in war. Chris Sherwell reports

THE search for a convention to cover unconventional weapons continues. The latest initiative has come from Britain in the sphere of chemical weapons, and, as the UK representative explained to the 720th plenary meeting of the CCD, combines new ideas and constructive and realistic elements from previous drafts into a draft treaty which is being presented as a focus for negotiation.

The 17-Article draft, which the CCD's 30 member states will study before their spring session in February next year, has several features. The emphasis on "confidence building" is one. Under Article II signatories would each establish or nominate a national verification agency; in declaring whether or not they were in possession of chemical weapons and producing figures they would, it is hoped, be generating the confidence without which the main provisions of the treaty would not come into effect.

Article III is also aimed at confi-

dence building. It amounts to a moratorium on production of the chemical weapons specified in a protocol still to be negotiated for Article I, by which signatories would have undertaken "never, in any circumstances, to develop, produce, or otherwise acquire, or use" chemical weapons or munitions or their delivery systems.

Another feature is the draft's three-stage implementation. After the first phase, in which signatories would stop production of chemical weapons and would provide information, the main provisions of the treaty would come into operation. The third stage, marked by Article VII, provides for the phased destruction or conversion to peaceful use of chemical weapons and the destruction of stockpiles. The exact programme is left for negotiation, but a Consultative Committee would arrange for the vital aspects of verification, inspection and exchange of information. Article IX offers a framework, which could also be amplified in a protocol.

Britain does not have any specific time scale in mind for the conclusion of a chemical warfare treaty, and with the summer session of the CCD about to end, is looking only for preliminary reactions. She has shown some eye for timing, however. Eastern bloc countries put up a draft convention in March 1972, as did Japan in April

1974; non-aligned countries had suggested a series of guidelines the previous year. In 1974 Canada suggested the phased destruction of all CW agents. This received the crucial support of the USA in April this year which, in the absence of the promised US-USSR initiative, made this the appropriate time for consolidation.

The time was also right because of technological improvements in monitoring techniques and because the experience of the International Atomic Energy Agency over nuclear safeguards showed that monitoring and protection of military and commercial secrets were not mutually exclusive. The US-USSR agreement earlier this year concerning on-site inspection of "peaceful" nuclear explosions is also regarded as an important breakthrough.

Whether Britain thinks member states now really believe that chemical weapons are unnecessary or that there is no need for stockpiles is another matter; the purpose of the latest proposal, however, is not seen as being to establish which countries are thinking along these lines by discovering their reactions to the draft. A readiness to move forward is apparently perceived, although it is acknowledged that the USSR might choose to describe any concessions it makes over verification in terms of "peaceful collaboration", rather than admit the change. □

COMECON

● The 30th meeting of Comecon in Berlin last month, held to assess the first joint five-year plan and discuss future integration, considered the prospect of integration over a fifteen to twenty year period in certain major sectors, including nuclear power. According to the joint communique of the meeting, there has been a "rapid growth" in the use of nuclear power during the past five years; the present capacity of nuclear power stations in the Comecon countries amounts to 7.5×10^6 MW, with an expected increase by 1980 to 30×10^6 MW, and Mr Kosygin in his address stressed the great importance of the "acceleration of the construction of nuclear power stations".

There have, however, been reports of the breakdown of Interatomenergo, the supranational Comecon body responsible for the construction of nuclear power stations, particularly since, shortly before the meeting, the Hungarian Deputy Premier Istvan Huszar strongly criticised the establishment of such supranational bodies whose policies may well run counter to those of individual members. In fact there seems to be no direct evidence that Interatomenergo (founded in December 1974) has broken down, although power supplies are, indeed, a pressing problem to Comecon.

About half, perhaps more, of the Comecon countries' electricity could be nuclear by 2000, but the short term solution cannot be a nuclear one; immediate plans for the exchange of power resources, which should in turn allow the reserve capacities of individual countries to be reduced, will for the moment be based largely on thermal power stations. Thermal power stations at present contribute some 87% to the total generating capacity of Comecon, and even with the planned nuclear expansion of the next five years, when nuclear power capacity will increase from 2.5% to 8% of the total, the role of the coal- and lignite-powered thermal stations will remain significant.

It seems doubtful that the Comecon countries will be able to fulfil all their long term plans without outside (that is, western) help, however, at least insofar as these plans depend on the development of more advanced reactors, such as the fast breeder, by the USSR. For the moment, though, the focus is on pressurised water reactors of the Voronezh type, which the USSR has developed commercially and can supply to its Comecon partners, including Cuba. And few in the West's nuclear in-

dustries doubt the benefits conferred by Comecon's unquantified but adequate uranium resources and by an equally unquantified but certainly unorganised anti-nuclear lobby.



● An agreement between the Comecon countries (including Mongolia and Cuba) on further cooperation in space exploration was signed in Moscow last month. According to Academician Anatolii Aleksandrov, President of the Academy of Sciences of the USSR, the agreement envisages cooperation in launching spacecraft, designing equipment, geological and meteorological surveying, joint technological research and the exchange of scientific data.

Such joint research has, of course, been taking place for some seven years now, under the Interkosmos programme. The latest two satellites in this series, Interkosmos 15 and 16, were launched on June 19 and July 27 respectively, and mark a new development in the programme—the monitoring of data from the satellites by member countries other than the USSR. Suitable receiver stations have already been built in Hungary, the GDR and Czechoslovakia, with Cuba and Bulgaria scheduled to join the monitoring network in the near future. The new Interkosmos satellites carry a special unified telemetry system developed jointly by Hungary, the GDR, Poland, Czechoslovakia and the USSR.

This stress on greater participation by the non-Soviet members of Comecon inevitably invites debate about the further development of the programme. Already there seems to be a trend for countries to specialise in individual fields of the programme. The data on solar radiation being

gathered in the current experiments is being processed by a Polish team in Torun, although it is also being assessed in Moscow by the Lebedev Physics Institute, which acts as coordinator. The GDR and Czechoslovakia appear to be especially concerned with the development of sophisticated electronic and optical equipment.

Such devolution and specialisation, if carried through, could well provide a new impetus to a programme in which, to date, one or two non-Soviet experiments at a time have been included in a still largely Soviet project.

The growing participation of member countries in the back-up research for manned space flights is another new feature of Comecon space research. The recent joint "Biosputnik" programme, according to a statement from Professor Stanislaw Baranski, Chairman of the Commission on Biology and Space Medicine of the Polish Academy of Sciences, has shown that three weeks in conditions of weightlessness "posed no danger" to the locomotor system or to muscle and bone tissue, but that the adrenal gland functions "revealed changes typical of stress". Similarly, the Hungarian Joliot-Curie Radiation Biology Institute has developed a model which absorbs various types of radiation in the same way as does the human organism. Although other, more conventional applications of this model are possible, notably in monitoring laboratory and industrial radiation hazards, its application to space research is especially emphasised.

This participation in space biology by the non-Soviet members of Comecon, which will continue with another "Biosputnik" project next year, raises the question of possible participation by Comecon crew members in Soviet space flights. Soviet space planning is greatly influenced by the concepts of Tsiolkovskii, who envisaged a large permanent orbiting space station as a *sine qua non* for any exploration deeper into space, and among the crew of several hundreds which he postulated for such a station, room for a token number of participants from Comecon member countries could presumably be found. The Soviet Academician Boris Petrov, Chairman of the Interkosmos Council, discussing the new Comecon space agreement, has meanwhile said that the Soviet Academy of Sciences is to hold talks in the near future with NASA about further cooperation in manned spaceflight.

Vera Rich

IN BRIEF

Immunisation clearance

A major hurdle to President Ford's \$130 million nationwide programme of immunisation against swine 'flu has been removed with the successful passage through Congress of legislation providing for individual claims for damages as a result of inoculations to be filed against the government. Mr Ford last week signed the bill introducing federal liability following threats of withdrawal from vaccine manufacturers themselves unable to arrange cover with private insurance companies. Under the new law injured parties would sue the government which could in turn sue programme officials accused of negligence or malpractice.

Marine conservation moves

Good news and bad for marine conservationists. The bad news came last week

when Britain, having lodged an objection with the North East Atlantic Fisheries Commission against continued overfishing of herring in international waters, itself decided to exceed the commission's recently-set catch quotas. Britain had earlier called for a complete ban on North Sea herring fishing, and only the Norwegian Prime Minister has asked his country's fishermen not to exceed existing catch limits when the exclusive economic zone extends to 200 miles.

The good news arrived with agreement between Brazil, Japan, South Africa and the USSR on catch figures for minke and sei whales in southern oceans over the next year. The new shares are based on reduced overall quotas set by the International Whaling Commission during its recent annual meeting in London.

Animal experiment proposals

A memorandum from a Parliamentary committee urging tighter controls over laboratory experiments with animals is now before the UK Home Secretary, Mr Roy Jenkins. The memorandum calls for new legislation more appropriate to modern experimental requirements, a more stringent system of licensing, and an increase in the number of government inspectors. It also urges an enlargement and greater powers for the Home Office Advisory Committee on Animal Experiments, which has met only 7 times in 10 years. The memorandum marks the launching of Animal Welfare Year and coincides with the centenary of the still current Cruelty to Animals Act. Last year saw a 3.3% drop in the number of experiments with living animals in Britain to just over 5.3 million.

PROGRESS in science depends on the communication of the results of research between scientists. There are those who think that the usual channels—the learned journals—do this inefficiently, and who propose novel techniques, but in practice the old methods continue. However, journals can only do their job if scientists submit papers describing their work, and if editors select good papers and reject bad ones.

This selection means that some sort of censorship operates, however much we may dislike the idea. Papers are refused because they are badly presented, or because their scientific content is considered unsatisfactory. Unfortunately many scientists cannot write clearly, and some ignore the instructions about presentation given by the journals to which they submit their work. Editors consider themselves justified in refusing badly written papers, but if the work described is good, they should try to encourage the author to resubmit an acceptable draft. Unfortunately few editors have the time to rewrite a manuscript, and therefore the results of some good work may be lost.

Fortunately for editors, in many university departments or research institutes the writings of junior workers are vetted by their senior colleagues, and this may ensure a proper standard of presentation and content. Generally this system works well, but occasionally the publication of valuable results is unnecessarily delayed by what amounts to internal censorship. There are those who are so obsessed by the bogey of premature publication that they always advise,

or even insist on, delay. At the same time many scientists find writing up their work to be tedious and difficult, and again they delay publication. Research workers who accept public money and then delay or do not publish their results could be con-

Censor judgment**KENNETH MELLANBY**

sidered guilty of fraud to the scientific community.

To judge the scientific merit of papers offered to them, editors usually rely on expert referees. Most referees take their duties responsibly, and make satisfactory assessments. Unfortunately some referees are prejudiced and recommend rejection of results contrary to their preconceptions; editors must be on their guard against this type of censor-

ship. Even with the greatest care, however, mistakes are made, as it may be hard to distinguish originality from idiocy. Outstanding scientists whose work is rejected may console themselves with the knowledge that, if it is good, recognition will not long be delayed. Unfortunately many of the unrecognised geniuses who complain that their work remains unpublished have little of worth to contribute to science.

The most controversial issue is the use of censorship on moral or ethical grounds. Most scientists consider that certain types of cruel animal experiments should be forbidden, and some journals have a policy of refusing to print articles describing such work. Papers describing what are considered to be unethical medical practices are also refused by a number of editors. At first sight this type of censorship seems to be justified, but has an editor the right to assume this "holier than thou" attitude? If the work is of scientific importance, or is likely to advance medicine substantially, has he a right to suppress it in this way?

If a research worker has done something which is illegal, or which contravenes the conditions of a vivisection licence, he should perhaps be warned of the possible consequences of the publicising of such acts. Editors may need to take legal advice regarding their possible liability for publishing such results. But scientific merit must surely remain the criterion by which scientific research and publication is judged. Unethical practices should be prevented, but not by censorship.

correspondence

Scientists in Argentina

SIR,—My intention in writing this letter is to present another point of view regarding the position of scientists in Argentina. The letter written by 70 Italian scientists (July 22, page 253) has indeed surprised me.

I can remember that a law identical to the one which the Italian scientists referred to (Ley de Prescindibilidad) was passed two years ago by the constitutional government of Argentina. Under that law the Peronist government "released from duty", for "reasons of service" with no further justification, thousands of public workers from both scientific and non-scientific institutions. It is funny that at that time this group of Italian scientists did not air complaints. In this previous instance the Minister of Education of the Peronist government established very clearly a policy inspired by the criterion "who is not occidental and Christian is subversive". He repeated this criterion in several public talks, and again at that time no complaints from this group of Italian scientists were heard.

If scientists are interested in the advancement of science and not in politics then they should be impartial in judging the internal political situations that affect a scientist's work, especially in South America where these problems very frequently arise.

Subversion in Argentina could be found in every department and institution of the government. Subversives are not only those who plant bombs; they are also those who support the "guerrilla" in different ways, those who steal public money and those who use power to their own benefit. Argentina is now fighting for its survival against these groups and fortunately it is winning. Of course there may be some unjustified cases in which an innocent person is "released from duty", but many more innocent people lost their lives in bombings and terrorist acts.

I am completely sure that among the 550 scientists (according to the Italian scientists' letter) who lost their jobs there might be a few innocent victims. These mistakes are unavoidable; but there are also many more who have used their public jobs for political activism. I know personally a number of scientists who in many years have not published a single paper describing their research. Apparently there was no research which suited their personal interest; only politics.

During these many years the taxpayers paid their salaries.

It is not true that leftist people are being pursued in Argentina. Terrorists and guilty people, disregarding their political preferences, are being pursued. Numerous rightist members of the last government are now in jail because of mismanagement of public funds and robbery.

Because of the economic disaster produced by the previous government scientific institutions had to cancel their subscriptions to practically all scientific publications. Since I am temporarily in the USA continuing my research, I am perhaps one of the few Argentinian scientists who can read *Nature* and other publications in my field of interest. My colleagues in Argentina are not so lucky.

It is very difficult for Italian scientists to imagine the real situation in my country in all aspects (not only science) over the past four years. For that reason I understand that it must be difficult for them to state an impartial opinion taking into account all the facts. Certainly it was an incorrect evaluation by these 70 Italian scientists that led them to write their uncritical letter to *Nature*.

HUGO LEVATO

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Alternative refrigerants

SIR,—F. A. Cotton (Correspondence, March 25, page 280) attributes to us the suggestion that, "if we do not choose to give up refrigerators, we may have to continue tolerating atmospheric pollution by chlorofluorocarbons, even if we ban aerosol cans". In fact, we made no such suggestion, as any reader can verify (Correspondence, March 4, page 8). Our letter dealt with facts only, and we suggested that any decision to ban R-12 take those facts into account.

Cotton states that "refrigerators . . . normally release their refrigerant gas only when junked". Actually, the reverse is usually true; refrigerators are junked once their moving parts are so worn that they can no longer retain the refrigerant for a reasonable length of time. For this reason alone, Cotton's suggestions for laws promoting the recovery of refrigerant from refrigerators would not solve the problem of chlorofluorocarbon refrigerant release to the atmosphere.

W. J. Megaw (Correspondence, May 6, page 261) says a short release time of two years for a refrigerant is partly due to freeze drying applications and partly due to head and tail collisions of automobiles containing air conditioning systems. A Bureau of Domestic Commerce study (*Economic Significance of Fluorocarbons*, December 1975) does not identify freeze drying as a significant source of fluorocarbon emission. The one- to two-year replacement time for automobile refrigerants is again due to leakage; automobile air conditioners are of necessity light weight and are subjected to vibrations and temperature extremes of the automobile engine. Automobile air conditioners use R-12 exclusively.

The BDC study attributes R-22 as being 30% of the total refrigerant used, not 50% as Megaw states. R-22 is used primarily in building air conditioning where low temperature is not required. Commercial and home refrigerators and freezers use R-12 almost exclusively. R-12 and R-22 are not interchangeable in existing refrigeration equipment, valued at \$100,000 million, because they have quite different physical properties. Total replacement of R-12 and R-22 would require redesigning many refrigeration systems. These refrigeration systems will necessarily be more expensive since they must operate at higher pressures. Difficulties posed by compressors burning out have, thus far prevented the use of R-22 in refrigerators and freezers. This design problem is a consequence of the high heat of compression, low density and high specific heat of R-22.

Model calculations by Sze and others indicate that an additional two years of fluorocarbon production will lead to 0.1% additional ozone reduction. This assumes an infinite troposphere lifetime for R-11 and R-12, that is, a maximum reduction in the ozone. We believe that an immediate ban on refrigerant or aerosol propellant uses of R-11 and R-12 would be unwise in view of present uncertainties in the theory. The two-year moratorium requested by industry to continue research programmes that began over a year ago, and which have already been fruitful, is very reasonable.

THOMAS J. LECK

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news and views

Assumed dipolar geomagnetic field

from Peter J. Smith

WHEN Johnson *et al.* (*Terr. Magn. atmos. Elect.*, **53**, 349; 1948) carried out their early palaeomagnetic work on recent Pacific sediments and the Holocene glacial clays of New England, they clearly saw their studies as just part of a much grander design. In respect of the wider aim their point was that "in order to determine the origin and nature of the Earth's magnetic field and to test the various hypotheses which have been advanced to explain the field, it is desirable to determine the history of this field throughout geologic time and to investigate more carefully its spatial variations, both inside and outside the Earth's surface".

As it turns out, it has been possible to make progress on the origin of the geomagnetic field largely without invoking the geological time dimension, and to this limited extent Johnson and his colleagues were mistaken. The self-exciting dynamo, the only viable field source known, may be regarded as an explanation of the present and recent fields without necessarily accepting that the details of any dynamo model applicable to today's field were valid throughout the Earth's history. Since the dynamo was discovered, detailed models have, of course, been extended to the geological time scale by the incorporation of field reversals and field intensity fluctuations, and for this and other reasons it is implicit in most modern models that the geomagnetic field has always been predominantly dipolar. But there is nothing in the dynamo principle as such that would preclude higher order fields in the past; and to the extent that more than the principle is applied to past ages, the dipolar nature of the field could be said to appear as an assumption.

It also appears as an assumption in most studies involving palaeomagnetic directions. Until the 1950s, palaeomagnetists generally felt that sufficient measurements would make it possible to define the morphology of the geomagnetic field throughout much of the Earth's life, subject only to the availability of enough rocks with sufficiently stable magnetisations. But ironically

they were to be thwarted in this by their greatest achievement, the quantitative proof of continental drift. Once it became clear that continents had moved, it became equally evident that no simple test for dipolarity based on a stationary frame of reference could exist beyond very strict limits. It would be impossible to separate easily the effects of definitely moving rocks from those of possibly changing field shapes.

So the dipole assumption now appears in two different situations, making it just as desirable to determine the history of the geomagnetic field as it was in the time of Johnson *et al.* But how can the dipole hypothesis be put to the test? As far as very recent times are concerned there is no problem, for movement of the reference frame resulting from drifting continents has been small and changes in palaeomagnetic pole positions on that account have been within the errors of measurement. Thus more than a decade ago, Irving (*Paleomagnetism and Its Application to Geological and Geophysical Problems*, Wiley, New York, 1964) showed that the eleven then-available poles covering approximately the past 7,000 yr were grouped around the present geographic pole, and McElhinny (*Paleomagnetism and Plate Tectonics*, Cambridge University Press, 1973) has shown that British archaeomagnetic poles taken at 100-yr intervals from AD 1900–1000 and AD 300–100 were distributed likewise. Both results suggest that during the past few thousand years at least the geomagnetic field has been dipolar and that on average the dipole lay along the rotational axis.

In practice, this test may also be successfully extended somewhat further back in time. According to McElhinny, for example, 67 poles covering the past 5 Myr are clearly grouped around the geographic pole with a mean pole at 88.8°N, 131.9°E. Opdyke and Henry (*Earth planet. Sci. Lett.*, **6**, 139; 1969) showed that palaeomagnetic inclinations from 52 deep sea sediment cores less than 3 Myr old were in close agree-

ment with those to be expected from an axial geocentric dipole. And Tarling (*Principles and Applications of Palaeomagnetism*, Chapman and Hall, London, 1971) has even shown that poles from igneous rocks up to 20 Myr old are grouped around the geographic pole, albeit with a greater scatter than for shorter, more recent periods.

Wilson and Ade-Hall (in *Palaeogeophysics*, Academic, New York, 1970), on the other hand, found a tendency for Quaternary and Upper Tertiary poles from individual regions to lie on their respective far sides of the geographic pole. On the face of it, this would seem to call in question the average axial nature of the dipole, although Wilson (*Geophys. J.*, **19**, 417; 1970) attributed the phenomenon to deviations from geocentricity; the axial dipole is apparently shifted northward with respect to the Earth's centre. In any event, the effect is small enough to be regarded as a second approximation. In no case is the basic dipolarity of the field in question; on the contrary, the dipole assumption is strengthened.

Beyond the Upper Tertiary, however, interference from continental movements causes difficulty. Over the longer geological period, poles of similar age from different regions of a single rigid landmass seem to be consistent with an average axial dipole, the variation of the dispersion of palaeomagnetic directions with palaeolatitude is usually comparable with that to be expected from the present dipolar field, and palaeolatitudes calculated on the axial dipole hypothesis are broadly consistent with latitude-dependent palaeoclimatic indicators. But none of these can be regarded as a rigorous test of dipolarity; the best that can probably be said is that there is little evidence actually to refute the dipole. The new test for dipolarity and its application to the Phanerozoic, reported by Evans on page 676 of this issue of *Nature*, is thus very welcome, although other tests may still be required before the persistence of the dipole can be said to be scientifically certain. □

It is not unreasonable to suppose that a predator normally reduces the numbers of its prey, but there are certain exceptions to this general principle. One example emerged from the experiments of Porter (*Nature*, **244**, 179; 1973) on the grazing of zooplankton upon planktonic algae. In a small mesotrophic lake in Connecticut she enclosed water samples in 0.5 m³ polythene bags and artificially enriched the population of zooplanktonic grazers within some of these. After four days Porter sampled the various bags and determined whether each of the phytoplanktonic taxa represented had decreased or increased in density.

Flagellates, nanoplankton and diatoms were suppressed by raised grazing pressures; desmids, dinoflagellates and chrysophytes were unaffected but, rather surprisingly, the larger green algae increased in density under more intense grazing. The unaffected groups were rarely found in the guts of the grazers; they were probably selectively avoided. The greens, particularly those species with a gelatinous sheath around their cells, were found to be viable after passing

Predator or prey?

Peter D. Moore

through the guts of grazing zooplankton. Porter suggested that the fragmentation of larger colonies of gelatinous greens in *Daphnia* guts could enhance population growth rates. She also considered it possible that the gelatinous sheaths could protect the cells against digestive enzymes and yet allow the cells to take up inorganic ions and small molecules even within the animal's gut.

Porter has now put these ideas to the test (*Science*, **192**, 1332; 1976) in a series of experiments involving radioactive tracers. The colonial alga *Sphaerocystis Schroeteri*, which has a mucilage sheath, was fed to the grazing crustacean *Daphnia magna*, together with the desmid *Ankistrodesmus falcatus* (no mucilage sheath) which had been supplied with NaH¹⁴CO₃ and K₂H³³PO₄. Subsequent sectioning and autoradiography of the *Daphnia* gut showed

that both ³³P and ¹⁴C had been taken up by *Sphaerocystis* from the broken cells of *Ankistrodesmus*. ¹⁴C uptake was enhanced by light, suggesting that its uptake was autotrophic. Thus nutrient supply, as well as the physical dissociation of algal colonies, may contribute to the observed enhancement in population growth rate. Porter has now been able to quantify the enhancement, for in a controlled experiment, the population of algae which had passed through a *Daphnia* gut had grown by 63% in 24 h. Ungrazed populations were unchanged in the same period.

Porter points out that the mucilaginous green phytoplanktonic taxa like *Sphaerocystis* have summer rather than spring blooms. At this time free nutrients in the water are depleted and the guts of grazers provide a locally enriched microhabitat. Porter refers to the relationship as one of 'nascent symbiosis' between aquatic plants and grazing animals. But it is certainly not a mutualistic one. It is a refreshing example of an evolutionary confidence trick whereby the hunter is exploited by its supposed prey.

How closely should continents fit together?

A RECENT article by Hallam (*News and Views*, **262**, 94; 1976) in which my paper on global expansion and continental displacement during the Mesozoic and Cenozoic (*Phil. Trans. R. Soc.*, **A281**, 223; 1976) is reviewed, contains certain factual errors which need to be corrected. Less important is his initial statement that I have argued post-Pangaea continental displacement to be a consequence of global expansion since the early Jurassic. This is not entirely correct, and the importance of the role of convection in the asthenosphere in the production of the Earth's oceanic crust is implicit throughout the paper. The continental geological information, oceanic-floor spreading patterns and the distribution of present and former subduction zones during the last 180 Ma indicate global expansion at the rate envisaged in my paper. These data do not support the concept of an Earth of constant modern dimensions, as progressive subtraction of isochronous oceanic crustal area on such a model readily shows.

Hallam cites three examples of areas of continental crust which occur in the magnetic quiet zones, and which might affect the fit of the continents together to reform Pangaea such as that given in my maps. The paper by Jansa and Wade (*Geol. Surv.*

Canada Pap., **74-(30)2**, 51; 1975) to which he refers actually describes the submerged continental shelf off Newfoundland including Flemish Cap. It is indeed continental crust and is shown as such on my maps. The adjacent Atlantic oceanic crust with its typical seismic and magnetic characteristics is well described by Grant (*Geol. Surv. Canada Pap.*, **74-(30)2**, 217; 1975) in the same volume. The magnetic quiet zone east of the Vöring Escarpment described by Talwani and Eldholm (*Bull. geol. Soc. Am.*, **83**, 3575; 1972) is also included in my maps as continental crust; the area to the west of it shows simatic crustal characteristics. The paper by Burolet and Byramjee (*Notes Mém. Comp. Fr. Pétroles*, **11**, 71; 1974) does not provide definitive evidence that widespread crustal areas off the continental shelf of Africa consists of downfaulted continental crust. The evidence available does indicate that east of the margin of the east African continental shelf, a thick sequence of sediments occurs probably extending back into the mid-Mesozoic as in the case of the eastern region of the Wharton Basin adjacent to the Australian continental shelf. The continental shelf off Tanzania shown on my maps has a sequence of sediments extending back into the

Palaeozoic (Kent and Perry (*Sedimentary Basins of the African Coasts* (Edit. by Blant, G.) **2**, 113; 1973) and Kent and Tarling (*Nature*, **261**, 304; 1976)). The examples given by Hallam, therefore, cannot be considered as convincing evidence against the major geometric problems considered in my paper, and indeed, the mounting geological evidence against the triangular Tethys Ocean required by constant dimensions reconstructions cannot be ignored (and this specific problem is recognised by Burolet and Byramjee (*op. cit.*)).

Hallam refers to the controversy about the original position of Madagascar in the Gondwanaland reconstructions. There is evidence that Madagascar reached its northern position relative to the eastern African margin in the early Mesozoic, having moved northward during the Palaeozoic in response to pre-Pangaea crustal movements. The evidence put forward by Flores (*Trans. geol. Soc. S. Afr.*, **73**, 1; 1970) for a position of Madagascar adjacent to South East Africa and Rhodesia—Mozambique during the deposition of the lower Karroo should be given careful consideration by workers interested in this area.

H. G. OWEN

How approximate are the laws of plate tectonics?

HALLAM (*News and Views*, 262, 94; 1976) has commented on a paper by Owen (*Phil. Trans. R. Soc.*, A281, 223; 1976) who put forward a case for very substantial recent expansion of the Earth. Both writers discussing this problem (and certainly the history of G is still a very open question, (Lewis, *Nature*, 261, 302; 1976)) appear to base their arguments on what Hallam terms a fundamental tenet of plate tectonics, "that the amount of crust created at ocean ridges must equal the amount subducted elsewhere". This is in fact, a law of constancy of mass of both oceanic and continental crust. The purpose of this comment is to point out that such a basic tenet cannot hold exactly for a body like the Earth which is mixing as it cools. A major problem of our science today is to find out just how approximate such laws really are. It also seems that if continents are to fit like pieces of a puzzle, there must also be laws of constancy of area and shape. Again, this cannot be exact for we know that the processes which lead to separation change thickness.

While at present we certainly cannot exactly quantify major earth-shell interactions, certain facts must be considered and I would here draw attention to a few examples:

(1) The rate of delivery of potassium to the oceans by solution processes alone is about 7×10^{13} g yr⁻¹. If such a value was typical throughout Earth history, then a mass of potassium equivalent to all that present in the continents would be delivered to the

oceans in about 5×10^8 yr. If we add the particulate contribution then the rate of removal of continents is much faster. As stated by Gilluly, Waters and Woodford (*Principles of Geology*, Freeman, 1975) "were the present rate of erosion for the United States maintained without further uplift or isostatic rebound, the country would be reduced to sea level in about 14 m.y."

(2) According to Sibley and Vogel (*Science*, 192, 551; 1976) "pelagic sediments, in contrast to other sediments, are not usually recycled; most are subducted and therefore act as a geochemical sink". Assuming some truth to this statement, then, as the rate of formation of pelagic sediments with about 3% potassium is about $1 \text{ km}^3 \text{ yr}^{-1}$, again this would subduct all continental potassium in 5×10^8 yr. The formation of such sediments involves the oceanic mixing process and a potassium atom from the Mississippi may well be subducted at the Chile-Peru trench.

(3) Low K basalt comes up and higher K spilite is subducted (Keen, *Geosci. Can.*, 2, 36; 1975). The surface layers of the ocean floor crust appear to average about 1% potassium. While there are many uncertainties (Fyfe, *Geosci. Can.*, 3, 82; 1976) potassium removal by way of spilite subduction seems in the same order of magnitude as that supplied to the oceans by solution.

(4) Low quartz andesites debris (graywacke) may well be subducted at appropriate sites by way of the re-

actions graywacke-glaucophane schist-quartz-phlogopite eclogite. We know little of this process except that we do know that graywackes are dragged to depths of at least 30 km to form glaucophane schists.

In summary, there is good evidence that the continents cycle through the oceans and back to the mantle at a rate that could change crustal balance. In the spreading process, leading edges (the Andes for example) thicken and trailing edges must suffer massive erosion, even by solution. During the motions there need be no perfect conservation of mass or shape of continental crust.

Finally, it should be noted that the present configuration of the Earth would not be stable for a cooler, mixed, body. In such an Earth, the hydrosphere would hydrate the mantle, and "continental" elements like K, Na, SiO₂, would form stable compounds like phlogopite and pyroxene in the mantle. Our crust and hydrosphere, even if subducted (Fyfe, *loc. cit.*) tend to be thrown back by thermal processes.

But if the Earth is cooling, there must be increased mantle fixation of crustal components because the Earth is mixing. If we allow that the combined influences of all the above mentioned processes, subduct the continental mass about each billion years, then we could lose 20% in 200 Myr. Perhaps the interesting fact is that the steady-state approximation of plate tectonic theory is rather good.

W. S. FYFE

Reply

HALLAM REPLIES: I AM pleased that my *News and Views* article should have provoked Fyfe into writing his interesting letter giving geochemical reasons why the 'basic tenet' that crust created equals crust destroyed cannot be exactly true. It is indeed apparent that continental crustal thickness must change when separation takes place; the remarkable fact is that computer-based 'jigsaw fits' are for the most part so accurate to a first approximation. I feel no need to make further comments but Owen's letter calls for a brief reply.

My purpose was less to review Owen's paper than to use it as an excuse to discuss some awkward anomalies in continental fits which have not been adequately acknowledged or discussed by Earth scientists. I cited the Jansa and Wade, and Talwani and Eldholm, papers as examples of detailed recent work undermining

the reliability of the geometric 500 fathom fit, with the implication that the same geological situation very probably holds for similar continental margins elsewhere in the world. The African margins are a good example, and absolutely crucial to reconstructions of Pangaea and to Owen's expanding Earth hypothesis. Yet Owen makes no allowance whatever for a broad zone of attenuated and subsided crust around this continent (see, for instance, his figure 11). The Burolet and Byramjee paper does not, admittedly, provide "definitive evidence", because it is in effect a broad-ranging review, and most of the critical data is based on unpublished work by oil companies. The paper does in fact contain substantial data for the region off Angola which cannot be lightly dismissed. Furthermore, Jansa and Wade spell out very clearly, with good evidence, that the north-west African

continental margin is virtually a mirror image of that off eastern Canada and the United States. As for Madagascar, it is not very helpful to cite once again the much discussed paper by Flores. His purely geological arguments for a southerly position of that island can be countered by other geological arguments for a more northerly fit. The point is that the arguments based on geological comparisons with mainland Africa have proved indecisive, and that is why the palaeomagnetic results of McElhinny and Embleton are so important.

In conclusion, I repeat that I find Owen's paper a stimulating one which deserves to be widely read, even though I am sceptical about his interpretation. If it serves to point up critical areas for further analysis it will have served a very useful purpose whatever one thinks of the conclusions.

A. HALLAM

Irreproducible results

from Robert M. May

CONSIDER the following game, played with the combinatorial theorist's mythical urn containing black and white balls. Initially the urn contains only two balls, one black and one white. Choose one ball at random from the urn, note its colour, and then replace it plus a new ball of the same colour as the chosen ball. The game thus proceeds, each round consisting of the random selection of one ball from the urn and replacement of this ball together with a new ball of the same colour. After N such rounds, the urn will contain a total of $N+2$ balls.

After many rounds, the urn will contain a large number of balls. What fraction of the balls will be white? The reader is invited to pause here, and to make a determined effort to guess the answer.

The answer is that for large N the proportion of white balls will tend to converge to some limiting value, p say; but this limiting value p is equally likely to take any value between 0 and 1. That is, when I play this game my plot of the proportion of white balls in the urn as a function of the number of rounds (or, equivalently, time) will exhibit some initial wiggles, but will eventually settle to a steady flat line corresponding to, say $p=0.391\dots$. When you play the game you see similar initial fluctuations, which damp out as the total number of balls gets large, and your experiment will similarly settle to some steady but different value of, say, $p=0.893\dots$, and so on. Each run of this experiment will yield to the experimenter an illusion of determinism, as his results settle to a steady limiting value; this value is, however, a random variable, differing from one experiment to the next, and determined largely by the statistical vagaries of the first few turns.

In short, this simple game provides an example where an underlying stochastic process gives results which appear deterministic (at least to the person who witnesses but one run). It is, in a sense, the mirror image of the phenomenon I recently reviewed (*Nature*, **261**, 459-467; 1976) whereby simple deterministic processes can produce results which appear indistinguishable from random fluctuations.

The above game has been known to mathematicians for some time, and has been discussed in relation to the spread of infection (Eggenberger and Polya, *Zeit. angew. Math. Mech.*, **3**, 279-289; 1923) and to stochastic population growth (Blackwell and Kendall, *J. appl. Prob.*, **1**, 284-296; 1964), but the basic

messages have tended to become buried under mathematical encrustations. Cohen (*BioScience*, **26**, 391-394; 1976), in a paper exuberantly entitled "Irreproducible Results and the Breeding of Pigs (or Nondegenerate Limit Random Variables in Biology)", has disinterred the phenomenon, and suggested that it may have widespread relevance in population biology.

Geneticists such as Robertson (*Proc. R. Soc. Lond.*, **B153**, 234-249; 1960) and Hill (*Biometrics*, **30**, 363-366; 1974) have realised that this urn-and-ball game captures some of the essential features of plant and animal breeding programmes, and that replicate lines from the same initial population can be very different in the limit they reach. Cohen suggests that similar "nondegenerate limits" may arise in many ecological contexts. For example, differences in successional sequences and in climax states in apparently similar habitats may not be due to causal differences between habitats, but may arise as variations in an ensemble of habitats (ultimately dependent on the statistical accidents of early colonisation). Differences in "species composition on apparently similar islands may result from the operation of identical forces which produce regularity only in an ensemble of islands. Differences in the sizes of prides of lions or in the social organisation of troops of Japanese macaques may reflect the inherently but lawfully variable outcome of identical underlying forces, rather than deterministic

ecological differences". Most of these suggestions have been made previously by other people, but not in so precise a form.

Turning to behaviour, Cohen suggests that it is "possible that some of the significant differences among mother-child interactions, which are obvious by the time a child reaches five years of age, are due neither to inherent differences among individuals nor to environmental differences, but to sequentially dependent random forces applying equally to all mother-infant pairs".

Cohen concludes on a metaphysical note. If we had been born in another universe which had started from exactly the same initial conditions as our present one and which had been subject to the same dynamics, would we necessarily infer the same laws of nature as we infer for this universe? The urn-and-ball game holds the suggestion that our present Universe may be an irreproducible result. □

Human trypanosomiasis today

from F. E. G. Cox

TRYPANOSOMIASIS has received a considerable amount of attention recently (see *Nature*, **249**, 677; 1974; **262**, 85; 1976 and the *Pan American Health Organisation Symposium on New Approaches to American Trypanosomiasis*, 1975) and the latest number of the *Transactions of the Royal Society of Tropical Medicine and Hygiene* (**70** part 2, 1976) contains a refreshingly summarised statement of the current status of this group of diseases under the title "Trypanosomiasis Today". Missing are the results of biochemical, ultrastructural and immunological studies that have made this field so attractive to many scientists over the past few years and instead there is basic information emphasising the need for sustained effort to be applied in the field and in the pharmaceutical laboratory.

In Africa, human trypanosomiasis, or sleeping sickness, is still widespread in the tsetse fly-infested areas where it exists in the form of unstable "controlled endemicity" (de Raadt, *Trans. R. Soc. trop. Med. Hyg.*, **70**, 114; 1976). Even in West Africa where the disease is under control in many places the number of new cases is increasing by about 10 per 100,000 people and there were 2,511 recorded new cases in Africa in 1974. This is a vast improvement on the 1970 figures of 9,140 but trypanosomiasis is still prevalent and

Bibliography of plant genetic resources

DURING the replacement of traditional crops by new varieties a great deal of the potential genetic variation present in the old varieties and their wild relatives has been lost. Over the past two decades however, interest in the conservation and use of the plant genetic resources that remain has been rapidly growing. The literature on the subject has naturally grown with it, and the first extensive bibliography of work in plant genetic resources has just been produced. It has been compiled by J. G. Hawkes, J. T. Williams and Jean Hanson of the University of Birmingham and as well as covering the literature devoted specifically to crop genetic resources, there is also extensive coverage of crop origins, variation and evolution, along with appropriate references in ecology, genetics, practical agriculture, phytopathology, and entomology.

The bibliography is produced by the International Board for Plant Genetic Resources and can be obtained from FAO, Rome.

the problem of "controlled endemicity" is that the movements of individuals and populations and any decrease in control measures are likely to bring about epidemics.

It is true that without control measures the situation would be worse than it is now and some of the success is due to the introduction of the drug pentamidine in 1949. No new drugs have been developed for field use for 20 years, however, (Williamson, *op. cit.*, 117). Pentamidine is only useful during the early stages of the disease and resistance to it has been recorded. The commercial production of other useful drugs is very restricted.

The immunological control of sleeping sickness is unlikely because humans seem to be unable to mount a good immune response to natural infections and because the parasites are capable of antigenic variation (Gray, *op. cit.*, 119) so control of the tsetse fly remains the best hope for eradicating this disease. The objective of insecticide control has been to eradicate the genus *Glossina* from a particular area and ground spraying has been quite successful, for example, in Nigeria, 27,500 km² of riverine area have been cleared of *G. morsitans* but in its natural habitat, the savannah, this species may present a different problem (Jordan, *op. cit.*, 128). The application of insecticides from the air is a relatively recent innovation and although the results are promising it is too early to evaluate this success. Without doubt insecticides will have to be used for some time to come while the possibilities of genetic and biological control are being investigated. Jordan points out that the tsetse flies are acting as conservation agents maintaining the *status quo* in Africa. Advances in trypanosomiasis control, tsetse fly eradication and land development will have to be carried out side by side if anyone is to benefit from such schemes.

Human trypanosomiasis in South America is called Chagas' disease and 35 million people are afflicted. Control of this disease is a major problem because man shares it with 100 species of wild mammals and up to 100 species of bugs that might transmit it (Minter, *op. cit.*, 124). Vector control, either by traditional insecticides or new methods, provides no immediate hope (Lumsden, *op. cit.*, 121) nor does chemotherapy. Only eight effective substances have been developed in 66 years (Gutteridge, *op. cit.*, 123) and of these only three are undergoing extensive tests. Lampit (5-nitrofurantoin) may soon be generally available but it is not completely effective and may have severe side effects. Possibly immunisation may be useful in the future because the parasite that causes Chagas' disease does

not undergo antigenic variation (Lumsden, *op. cit.*). Immunisation is only a remote possibility, however, but with only three companies testing compounds against Chagas' disease and with insecticides such as DDT becoming

scarce and expensive the immediate prospects of eradicating the disease do not look good. The increasing scarcity and expensiveness of insecticides is also a problem in attempts to eradicate sleeping sickness in Africa. □

Spiral galaxies at Cambridge

from Vincent Icke

About 35 astronomers interested in the structure of spiral galaxies discussed their findings and frustrations during a study week at the Institute of Astronomy, Cambridge.

SPIRAL galaxies are common, as Donald Lynden-Bell (Institute of Astronomy, Cambridge) pointed out in his introductory talk; considering the extremely slow evolution of galaxies, this implies that spiral structure in one form or another is almost as old as the Universe. To be explained are, among other things: why spirals occur in disk galaxies only; why spiral galaxies always contain interstellar gas; why any one form of spiral occurs, and why some spirals have bar-shaped inner parts. Recalling that it seems to be very difficult to stabilise a disk of stars against a bar-like gravitational instability, Lynden-Bell proposed that such a bar does, in fact, develop, and subsequently generates a spiral-shaped shock wave in the interstellar gas. After this introduction, contributions along six main routes followed.

The problem of the linear stability analysis of a differentially rotating disk of self-gravitating point particles (stars), has been haunting theorists especially after Lindblad's pathfinding attempts to explain galactic spirals as a normal mode of oscillation. Alar Toomre (Massachusetts Institute of Technology) described the analysis of a disk wherein all stars have the same mean orbital velocity. In such a disk, the random velocities of the stars as expressed in their distribution function (a solution of the Vlasov equation) assume a manageable analytic form. With his coworker Zang, Toomre discovered that $v = \text{constant}$ disks are, surprisingly enough, stable with respect to two-armed instabilities, but, an even greater surprise, strongly unstable with respect to one-armed spiral perturbations! Quite the opposite has been found for disks where the rotation curve goes to zero in the centre, as is observed in spiral galaxies. In an impressive piece of analysis, Agris Kalnajs (Mt Stromlo and Siding Spring Observatories) concluded that this inner region of solid-body rotation (where velocity is proportional to radius) is

largely responsible for the instability. The usual procedure to avoid instabilities is to reduce the effective mass of the stars, by, for example, surrounding the disk with a massive halo. Kalnajs showed that it is sufficient to introduce a 'nuclear bulge', that is, a small halo near the galactic centre where the rotation curve follows the solid-body line. A decrease of a factor of 4-5 in the growth rate of the instabilities was readily obtained in this way. Introduction of a bulge or halo effectively means the assumption of a vertical dimension in the disk, and Douglas Heggie (Royal Observatory, Edinburgh) showed that this may lead to resonant interaction between horizontal and vertical stellar motions. Approximating the stellar orbits as a three-dimensional Lissajous figure, he calculated that 1-4% of all stars may participate in resonances that bring them as far as half the disk radius out of the plane.

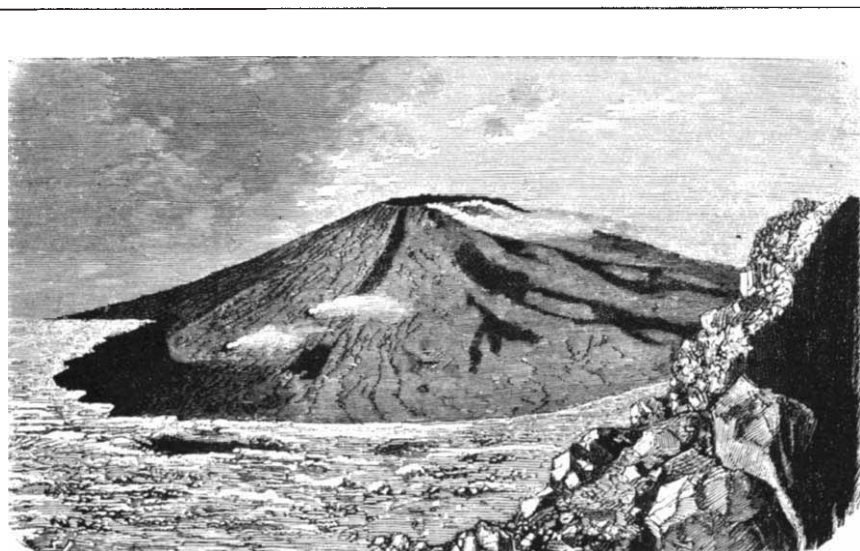
Linear instabilities attempt to grow indefinitely, but are ultimately stopped by non-linear effects. The analysis of these entails an awesome amount of mathematical physics, and has therefore barely begun. Elly Dekker (Sterrewacht te Leiden) described her application of quasi-linear techniques borrowed from plasma physics to the evolution of the particle distribution function in a stellar disk. Assuming that the potential can be split in a large, slowly varying, and a small, rapidly varying part, Dekker derived a diffusion equation for the evolution of the mean particle distribution. If the perturbation is stationary in a corotating frame, the diffusion coefficients depend on the contributions from the resonant stars only. She also demonstrated that the distribution function evolves so as to increase the coarse-grained entropy associated with it.

Bar-shaped distributions of stars are dynamically even more complex than disks. Some equilibrium models have been derived, and Scott Tremaine (California Institute of Technology) analysed the particular family that is obtained by squashing a homogeneous tri-axial ellipsoid along its rotation axis to zero thickness. He found that these bars are unstable if they are nearly

circular or rotating very rapidly. The remaining configurations are stable, at least up to third-order harmonic deformation. Apparently, rather elongated bars can survive when rotating slowly.

Increasing computational power has facilitated numerical studies of gravitating N -body systems. The main problem remains the simultaneous modelling of the long and the short range forces. Special techniques are needed to approximate both close encounters and global structure in such a way as to preserve the collisionless behaviour of the system. Robert Berman (University of Reading) gave a progress report on a potential-solving technique developed by Hockney and Brownrigg for a fully three-dimensional 25,000-body system. Studies of disk-halo models with halo potentials more realistic than those hitherto used, showed that the disk can be stabilised against bar formation if the halo is only marginally more massive than the disk. Also, if bars did develop, they often showed structure in their interior, in the form of nested rings or a smaller bar.

The considerable mathematical difficulties arising in the attempt to solve the Vlasov equation naturally lead to a search for shortcuts, preferably by assuming that the system can be described as a gas or similar continuum. An especially successful dodge was employed by Jim Bardeen (University of Seattle) to determine the unstable modes of a gaseous disk. He expanded the potential to first order in the disk thickness, and wrote the resulting equations in terms of Legendre polynomials. This enabled him to reduce the stability problem to a matrix eigenvalue equation. Truncating the matrix to 96×96 , a set of growing modes emerged with some interesting properties. An increase in gas pressure decreased the number of growing modes, but did not reduce their growth rates very much. The potential of the modes was not a simple multiple of their surface density, unlike the WKB modes studied by others. Most significantly, the modes appeared to be superpositions of short-wave partial modes travelling inwards and outwards. The flow of gas induced in the plane of a bar-like potential depression was studied by Karl-Juhan Donner (Institute of Astronomy), who followed up Lynden-Bell's earlier suggestion, and by Takuya Matsuda (University College, Cardiff), who in collaboration with Sorensen and Fujimoto employed a 50×30 point fluid-in-cell method to obtain a numerical simulation of the gas flow in a barred spiral galaxy. Matsuda showed that standing shocks develop roughly parallel to the bar, through which gas is deflected so strongly that it rapidly



A hundred years ago

The Inclosure and Cone of the present Crater: The Volcano of Réunion. from *Nature*, 14, August 17, 335; 1876.

assembles in the galaxy's nucleus. A second pair of shocks, detached from the first set, occurred near the region where the Keplerian velocity in the galactic plane equals the rotation velocity of the bar. The behaviour of motions out of the galactic plane, induced by shocks in the disk, was calculated by Alastair Nelson (University College, Cardiff), who found that the perturbations propagated almost vertically upwards, reaching considerable amplitudes in the tenuous regions at 300 pc height.

The last word, obviously, was for the observers, who produced something like a fascinating cold shower. Renzo Sancisi (Sterrekundig Laboratorium 'Kapteyn', Groningen) produced new radio maps obtained with the Westerbork synthesis radio telescope of NGC5383, the best maps of a barred spiral obtained to date. Gas was found concentrated near the ends of the bar and especially in the nucleus. Also, a low-brightness disk extending over a diameter of fully three disk lengths was observed, which made it possible to determine the rotation curve in this region. It appears that the central parts (out to half the length of the bar) rotate like a solid body. Thereafter the rotation speed stays almost constant. The bar was also seen in 1,415 MHz continuum, and deep plates taken by Van der Kruit and Bosma show that the optical extent of the galaxy is about as large as the neutral hydrogen disk. Evidence that even nicely-looking galaxies like M81 can be involved in spectacular events was produced by Leonid Weliachew (Observatoire de Paris, Meudon) and by Geoffrey Cottrell (Mullard Radio Astronomy Observatory, Cambridge) who described

observations of the region around M81, M82 and NGC3077. These are surrounded by a huge hydrogen halo, in which the velocities of the gas clouds link up nicely with those of the galaxies. Cottrell presented a computer model of a tidal encounter between M81 and NGC3077, which properly reproduced the radio appearance of the latter. Presumably the hydrogen halo consists of debris left over from an encounter between M81 and M82. Finally, it was demonstrated that many spiral galaxies are not flat at all, perhaps necessitating some drastic rethinking of extant models. Darrell Emerson summarised an observational programme of the MRAO on M33 and M31, both members of the Local Group of galaxies. The plane of M33 was known to be warped, and the new observations conclusively showed the enormous extent of these distortions, with warps rearing above the plane to heights almost equal to the galaxy's radius. In M31, the Andromeda Nebula, smaller warps were discovered at the edge of the observable radio disk (about 30 kpc radius). Then Sancisi proceeded to show that possibly all spiral galaxies are warped: four members of a nearby, but otherwise random, sample of five spirals seen almost edge-on showed up to 20% warping of their planes. Two of these have no nearby companions, so that if the warps are due to tidal distortion during an encounter between two galaxies, the distorted galaxy must maintain a rather crisp warp during 4-5 galactic years at least. This baffling finding left the participants in the discussion appropriately wondering how much progress, exactly, has been made in understanding spiral structure. □

articles

Dynamo model of double radio sources

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A model of radio sources is proposed in which the magnetised accretion disk of a massive black hole acts as an electric dynamo producing two oppositely-directed beams of ultra-relativistic particles.

WE give here a brief development of a model of double radio sources with the conducting, magnetised accretion disk of a massive black hole acting as an electric dynamo. The electric field created by the rotating disk results in the steady generation of two oppositely-directed, collimated, tenuous beams of ultra-relativistic protons. The output power in the beams is $\sim 10^{44}$ erg s $^{-1}$ and the proton energies are $\lesssim 10^{19}$ eV for a poloidal magnetic field of the disk of 10^3 gauss and a black-hole mass of

$10^8 M_\odot$. The beams propagate parallel to the angular momentum vector of the disk. The energy transported by the proton beams is gradually degraded because instabilities lead to the sharing of the beam energy with the ambient plasma. It seems possible that the two-stream interaction can convert the energy of a tenuous proton beam into a relatively dense, collimated beam of relativistic electrons. Energy carried by the electron beams is released as synchrotron radiation in the radio components.

With this model it seems possible (1) to have a large, steady supply of energy to widely separated small radio components, (2) to obtain alignment and symmetry of the two radio components, and (3) to have a correlation between the axis of the radio components and the direction of the angular momentum of the parent galaxy. The model includes some of the features of the 'twin exhaust' model¹, the spinar or oblique-rotator models²⁻⁵, the magneto-hydrodynamic models^{6,7} and the axisymmetric pulsar model⁸. The isotropic production of low energy ($\sim 10^{12}$ eV) cosmic rays from the accretion disk of a massive black hole has been discussed previously^{9,10}.

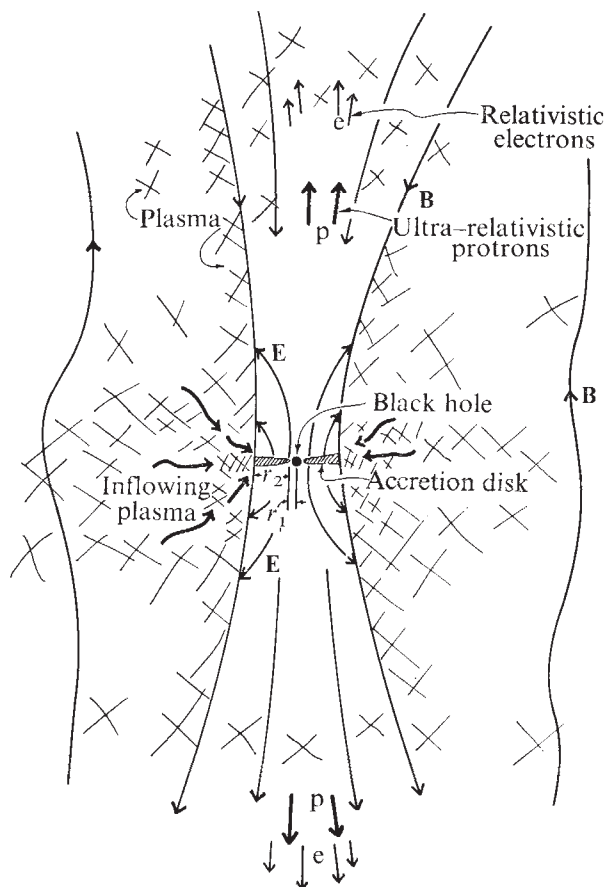
Model

Consider the possibility^{9,10} that a black hole of mass M_h ($\sim 10^8 M_\odot$) has formed in the centre of a galaxy (or in the nucleus of a quasar) and that the black hole is surrounded by a massive, flat accretion disk (see Fig. 1). Gaseous material accreted on to the disk is supplied from the mass loss of stars, possibly increased by disruptive collisions between stars and/or the close approach of stars to the black hole¹¹. It is evident that the angular momentum vector of the disk will have a direct correlation with the angular momentum vector of the parent galaxy. If the mass of the disk is sufficiently large, $M_d \gg M_\odot$ (but $M_d \ll M_h$), then the angular momentum of the disk may have a fixed direction in space for long periods, greater than the lifetime of a radio source. For $M_d \gg M_\odot$, the disk angular momentum corresponds to an average of the angular momenta of a large number of stars.

The magnetic field

Ionised matter moving into the accretion disk will carry with it a weak ambient magnetic field. The gradual inward radial motion of matter in the disk will amplify the field, tending to establish $B_z(r) \propto \sigma(r)$, where σ is the surface density of the disk, r is the radial distance from the black hole, and the z axis is normal to the plane of the disk. We consider that B_z at some particular epoch has a single polarity throughout the inner part of the disk. Because of differential rotation of the disk, the poloidal field ($B_r, 0, B_z$) is expected to be axisymmetric. It is, however, possible that the poloidal field is augmented by non-axisymmetric turbulent fluid motions¹² in the disk. Reconnection of the poloidal field lines occurs in the region $r < r_1, |z| < r_1$ (where r_1 is the inner radius of the disk) so that there is no build up of flux through the surface $r < r_1, z=0$. (For the moment,

Fig. 1 Schematic drawing of the electric dynamo formed by a conducting magnetised accretion disk. The radio wave emitting regions are discussed in the text.



consider that the space above and below the disk is a vacuum. Thus, although there may be a toroidal field (B_ϕ) embedded in the disk^{9,10}, $B_\phi = 0$ in the space above and below the disk.)

The electric field

The material of the disk may be assumed highly conducting so that an observer in a non-rotating (inertial) reference frame sees a strong radial electric field, $E_r(r) = -[u_\phi(r)/c]B_z(r)$, on the top and bottom surfaces of the disk, where $u_\phi = (GM_h/r)^{1/2}$ is the Keplerian velocity, assumed much larger than u_r . Thus the potential difference across the disk is

$$V_{12} = -\frac{1}{c} \int_{r_1}^{r_2} dr u_\phi(r) B_z(r) \quad (1)$$

where $r^1 = 6GM_h/c^2 \simeq 10^{14}$ cm ($M_h/10^8 M_\odot$) is the inner radius of the disk, and $r_2 \gg r_1$ is the effective outer radius where material enters the disk. If we assume $u_r(r) = \epsilon u_\phi(r)$, with $\epsilon = \text{a constant}$, $\ll 1$, then $\sigma \propto r^{-1}$. Because $B_z(r) \propto \sigma(r)$, we find

$$V_{12} \simeq -\sqrt{(6)B_*(GM_h/c^2)} \ln(r_2/r_1) \quad (2a)$$

where $B_* = \max(B_z) = B_z(r_1)$. In practical units

$$V_{12} \sim 10^{10} \left(\frac{B_*}{10^3 \text{ gauss}} \right) \left(\frac{M_h}{10^8 M_\odot} \right) \ln \left(\frac{r_2}{r_1} \right) \text{ V} \quad (2b)$$

Observe that V_{12} is proportional to r_1 , which is well defined, but depends only weakly on r_2 .

Magnetic flux and time scales

The magnetic flux through the disk is

$$\Phi \sim 4 \times 10^{31} \left(\frac{B_*}{10^3 \text{ gauss}} \right) \left(\frac{M_h}{10^8 M_\odot} \right)^2 \left(\frac{r_2}{r_1} \right)^{3/2} \text{ gauss cm}^2 \quad (3)$$

For $B_* = 10^3$ gauss, $M_h = 10^8 M_\odot$, and $r_2/r_1 = 10$, this flux ($\sim 10^{33}$ gauss cm²) corresponds to an ordered field of 10^{-6} gauss spread over a region of ~ 10 pc. A characteristic time scale for rapid dynamical variations of the disk is the orbital period at $r = r_1$, $T_d \simeq 5 \times 10^4 (M_h/10^8 M_\odot)$ s. At the other extreme, the time scale for significant changes in the large scale magnetic field structure, once it is established, is $T_B = \mathcal{L}/R$, where $\mathcal{L}$ is the inductance and R the resistance of the configuration. We estimate that T_B is at least as long as the lifetime of the disk because of the large size and high conductivity of the disk.

Resulting particle acceleration

Consider the particle acceleration resulting from the disk potential. In the space outside the cylinder $r > r_2$, there is assumed to be plasma, possibly in turbulent motion, but not in rapid rotation. The electrical potential of this plasma may be taken to be zero. The region $r < r_2$ above and below the disk is for the moment assumed a vacuum. We consider in the present study only the case where B_z is anti-parallel to the angular momentum vector of the disk. Thus the potential on the disk surfaces, $V(r, \pm 0)$, produces a vacuum field in the space above and below the disk. That is, $\nabla^2 V = 0$, with $V(r, \pm 0)$ specified, with $V(r_2, z) = 0$, and with $V(r, |z| \gg r_2) = 0$. The potential $V(r, z)$ implies an electric field which tends to accelerate protons (and other ions) in the $+\mathbf{z}$ ($-\mathbf{z}$) direction out of the top (bottom) surface of the disk. The electric field, E , is $\sim (u_\phi/c)Bz \lesssim 10^5$ V cm⁻¹ for $B_* = 10^3$ gauss and $M_h = 10^8 M_\odot$. The electric force on a proton exceeds the gravitational attraction of the black hole by a large factor, $\sim 10^{12}$. The electric field is, however, small compared with the poloidal magnetic field, $E^2/B_z^2 \sim u_\phi^2/c^2 \ll 1$.

The self-consistent, space-charge-limited flow of protons off the disk surface follows from general dimensional considerations (ref. 13 and E. Ott, T. Antonsen, and R. Lovelace, unpublished) in that the particle motion is extremely relativistic. If I denotes the total current of protons in, say, the $+\mathbf{z}$ direction, then

$$I = \kappa c V_{12} \quad (4a)$$

where κ is a dimensional factor which depends on the geometry through $V(r, \pm 0)$. We may assume that κ is not greatly different from unity (ref. 13 and E. Ott, T. Antonsen and R. Lovelace, unpublished). An estimate analogous to equation (4a) occurs in the axisymmetric pulsar model⁸. Using equation (2b), we rewrite equation (4a) as

$$I \sim 3 \times 10^{17} \kappa \left(\frac{B_*}{10^3 \text{ gauss}} \right) \left(\frac{M_h}{10^8 M_\odot} \right) \ln \left(\frac{r_2}{r_1} \right) \text{ A} \quad (4b)$$

Charge neutrality of the disk is maintained by a non-relativistic electron current in the region $r > r_2$. For $r < r_2$ and $|z| > 0$, there is a positive space-charge density J_z/c , where $J_z(r)$ is the beam current density.

The output power in the two beams is, from equations (2b) and (4b)

$$L_R \simeq 2IV_{12} \sim 10^{44} \kappa \left(\frac{B_*}{10^3 \text{ gauss}} \right)^2 \left(\frac{M_h}{10^8 M_\odot} \right)^2 \left[\ln \left(\frac{r_2}{r_1} \right) \right]^2 \text{ erg s}^{-1} \quad (5)$$

The power in the beams derives from the infall of matter into the gravitational potential of the black hole. There is a corresponding flow of angular momentum⁸ out of the disk carried by the poloidal-magnetic field and self-electric field of the beam and the beam protons. The beam emission may, however, represent only a small dynamical influence on the disk if $L_R \ll L_{\text{tot}}$, where L_{tot} is the total luminosity of the disk. In particular, an irregular magnetic field embedded in the disk may be dynamically important^{9,10,14}. For comparison, the Eddington limit on the electromagnetic emissions of the disk is $L_E \sim 10^{46} (M_h/10^8 M_\odot) \text{ erg s}^{-1}$.

Evidence in support of equations (4) and (5) comes from many laboratory experiments which have demonstrated the production of high-current electron beams^{15,16} ($I_e \sim 10^5$ A) and ion beams (P. Dreike, C. Eichenberger, S. Humphries, and R. Sudan, Cornell University Laboratory of Plasma Studies Report 172 (1975)) ($I_p \sim 10^4$ A) in this type of field configuration¹⁵⁻¹⁸ ($E^2/B_z^2 < 1$) at low voltages ($V_{12} \lesssim 10^6$ V) with electric fields $E \lesssim 10^6$ V cm⁻¹. The observed beam particle energies are $\sim \text{eV}_{12}$.

Beam energy/magnetic energy

An important dimensionless parameter for the dynamics of the beams is the ratio, denoted β , of the kinetic energy density of the beam to the energy density of the magnetic field. We first estimate $\beta = \beta_0$ near the disk, $|z| \lesssim r_2$. Note that we have a limit on the proton Lorentz factors γ from equation (2b)

$$\gamma \leq \gamma_0 \sim 10^{10} \left(\frac{B_*}{10^3 \text{ gauss}} \right) \left(\frac{M_h}{10^8 M_\odot} \right) \ln \left(\frac{r_2}{r_1} \right) \quad (6)$$

The spectrum of γ values as a function of r is expected to be relatively flat for $\gamma < \gamma_0$. An estimate of the beam density, n_B , follows from equation (4b)

$$n_B \sim 2 \times 10^{-3} \kappa \left(\frac{r_1}{r_2} \right) \ln \left(\frac{r_2}{r_1} \right) \left(\frac{B_*}{10^3 \text{ gauss}} \right) \left(\frac{10^8 M_\odot}{M_h} \right) \text{ cm}^{-3} \quad (7)$$

The total number of protons emitted per second is thus $\sim 4 \times 10^{38}$ for $n_B = 2 \times 10^{-5} \text{ cm}^{-3}$, $r_1 = 10^{14}$ cm, and $r_2/r_1 = 10$.

From equations (6) and (7), we find

$$\beta_0 = 8\pi \frac{n_B \gamma_0 m_p c^2}{B_z^2} \sim 0.7\kappa \left[\frac{r_1}{r_2} \ln \left(\frac{r_2}{r_1} \right) \right]^2 \quad (8)$$

where m_p is the proton rest mass. Evidently, we have $\beta_0 \ll 1$ if $r_2 \gg r_1$. That $\beta_0 \ll 1$, is a result of the fact that $u_\phi^2/c^2 \ll 1$ over most of the surface area of the disk. Thus for the proton acceleration and streaming in the vicinity of the disk, the magnetic field B_z is very strong. To a good approximation the protons follow the magnetic field lines and the configuration is force-free. (Values of β small compared with unity are known to be favourable to the magnetohydrodynamic stability of beams electrically neutralised by plasma²⁰.)

In the vicinity of the disk there is a small toroidal self-field B_ϕ from the proton beams. We estimate that

$$\frac{B_\phi}{B_z} \simeq \frac{2I}{cr_2 B_z} \sim (\beta_0 \kappa)^{1/2}$$

is small compared with unity if $\beta_0 \ll 1$. This toroidal magnetic field counteracts the radial space-charge repulsion of the beam and thus tends to collimate the beam.

Divergence of beam

For distances $|z| \gg r_2$ above and below the disk, the proton beams diverge. The outer beam radius is approximated as $a(z) = r_2 (1 + |z|/H)$, where H is the scale height for the divergence. We assume that processes involved in the initial outward propagation of the beams give $H \gg r_2$. Because the beam particles start off following the field lines, $B_z(z) \propto [a(z)]^{-2}$. The β factor of the beam is proportional to a^2 , and, therefore, we have $\beta \simeq 1$ at a distance $|z| \simeq z_* \equiv H(\beta_0)^{-1/2} \gg H \gg r_2$, where β_0 is given in equation (8). For distances $|z| \gg z_*$, where $\beta \gg 1$, the beam divergence comes from the ballistic motion of the protons (in the absence of instabilities). The usual adiabatic energy losses, involved, for example, in the expansion of a cloud of particles, do not occur for a particle beam in ballistic motion.

Radiation effects

In the initial outward propagation of the beam there may be significant curvature radiation. If ρ denotes the instantaneous radius of curvature of a proton trajectory, then the typical energy of the curvature photon is

$$\left(\frac{3}{2} \right) \gamma^3 \left(\frac{\hbar c}{\rho} \right) \sim 3 \times 10^{10} \left(\frac{\gamma}{10^{10}} \right)^3 \left(\frac{10^{15} \text{ cm}}{\rho} \right) \text{ eV}$$

The rate at which a proton emits energy is

$$\frac{d\gamma}{dz} = \frac{2r_e}{3} \left(\frac{m_e}{m_p} \right) \frac{\gamma^4}{\rho^2}$$

where r_e is the classical electron radius, and m_e is the electron rest mass. Thus the path length for a proton to lose half its initial energy ($\gamma m_p c^2$) is

$$l_{\text{cur}} \sim 2 \times 10^{16} \left(\frac{10^{10}}{\gamma} \right)^3 \left(\frac{\bar{\rho}}{10^{15} \text{ cm}} \right)^2 \text{ cm}$$

where $\bar{\rho}$ is an average of the radius of curvature. In the initial stages of formation of a source we may have $l_{\text{cur}} \lesssim \bar{\rho} \simeq r_2$ if $B_* M_h \gtrsim 10^{11} \text{ gauss } M_\odot$. One effect of the curvature photons may be that of expelling matter and thus straightening the magnetic field in two conical regions of small solid angle defined by the velocity distribution of the protons at $|z| < r_2$. The effect is, however, inefficient because the production and scattering cross sections for the curvature photons interacting with electrons

and protons are small ($\lesssim 2 \times 10^{-26} \text{ cm}^2$). The curvature photon production of electron-positron pairs off the magnetic field²¹ is negligible.

The beam protons may interact with low energy photons²² emitted by the accretion disk^{9,10} and surrounding plasma to give pions and electron-positron pairs. The proton energy threshold for pion production is

$$\sim 10^{17} (1 - \cos \Theta)^{-1} \left(\frac{1 \text{ eV}}{\mathcal{E}} \right) \text{ eV}$$

where Θ is the angle between the proton and photon momenta, and $\mathcal{E}$ is the average photon energy. The threshold for e^-/e^+ production is a factor 1/135 smaller. We estimate that the proton energy loss is small if

$$L_{\text{ph}} < 10^{46} \left(\frac{\mathcal{E}}{1 \text{ eV}} \right) \left(\frac{R}{10^{17} \text{ cm}} \right) \text{ erg s}^{-1}$$

where L_{ph} is the photon luminosity, and R is the effective radius of the photon-emitting region.

Counter flow instability

For distances $|z|$ larger than a certain value z_{neut} (discussed below), the proton beams become both charge and current neutralised by plasma in the beam channels. In the outward propagation of a beam, plasma enters the beam front, and, furthermore, plasma may leak radially into the beam channel because of instabilities (particularly for $|z| > z_*$, where $\beta > 1$). Thus the presence of free electrons on magnetic field lines connecting with the accretion disk presents the possibility of electrons counter-flowing and partially 'shorting-out' the proton accelerating potential. This shorting would result in an ohmic dissipation (and radiation) of a fraction ($\sim 1/2$) of the power $2IV_{12}$ in a small region of size $\lesssim r_2$ centred on the black hole with the remaining fraction of the power in the two proton beams. There is, however, a definite asymmetry between the proton acceleration and the counter acceleration of electrons.

Consider the two-stream interaction: let n_p denote the density of cold electrons (speeds $\ll c$) within the beam channel at some distance z . Then the dispersion relation for electrostatic waves with frequency ω and wavevector $\mathbf{k}$ is

$$0 = 1 - \frac{\omega_p^2}{\omega^2} - \frac{\omega_B^2 \psi^2}{(\omega - k v_B \cos \psi)^2} \quad (9)$$

where $\mathbf{k}$ is at an angle ψ to the beam velocity $\mathbf{v}_B$, with $\gamma^{-1} \ll \psi \ll 1$, and where $\omega_p = (4\pi e^2 n_p / m_e)^{1/2}$, $\omega_B = (4\pi e^2 n_B / \gamma m_p)^{1/2}$, $k = |\mathbf{k}|$, and $v_B = |\mathbf{v}_B| = c(1 - 1/2\gamma^2)^{1/2}$ is the local proton speed. It is evident from equation (9) that the proton beam may be considered weak in the limit where $n_p \gg (m_e / \gamma m_p) n_B \psi^2$. In this limit a simple form of the two-stream instability occurs²³. The frequency of the unstable wave is written as $\omega = \omega_p + \delta$, with $|\delta| \ll \omega_p$, and $k = \omega_p (v_B \cos \psi)^{-1}$ so that

$$\text{Im}(\delta) = \frac{\sqrt{3}}{2} \left(\frac{\omega_B^2 \omega_p}{2} \right)^{1/3} (\psi)^{2/3}$$

or

$$\text{Im}(\delta) \sim 3 \times 10^{-5} \left(\frac{10^{10}}{\gamma} \right)^{1/3} \left(\frac{pc}{a} \right)^{2/3} \left(\frac{n_p}{10^{-3} \text{ cm}^{-3}} \right)^{1/6} \psi^{2/3} \text{ s}^{-1} \quad (10)$$

where we have taken

$$n_B = 10^{-12} \left(\frac{pc}{a(z)} \right)^2 \text{ cm}^{-3}$$

with $a(z)$ the beam radius. The characteristic wavelength of the unstable wave is

$$\lambda \simeq \frac{2\pi c}{\omega_p} \sim 10^8 \text{ cm} \left(\frac{10^{-3} \text{ cm}^{-3}}{n_p} \right)^{1/2}$$

The phase velocity of the unstable wave

$$v_\phi = c \left[1 - \frac{\psi^2}{2} - \frac{1}{2} \left(\frac{\omega_B^2 \psi^2}{2\omega_p^2} \right)^{1/3} \right] < v_B \quad (11)$$

is quite close to the speed of light. As an illustration of equation (10), for $a = 10^{-2} \text{ pc}$ ($= 3 \times 10^{16} \text{ cm}$), $\gamma = 10^{10}$ and $n_p = 10^{-3} \text{ cm}^{-3}$, an e-folding of a wave with $\psi = 10^{-3}$ occurs over a distance of $\sim 10^{-3} \text{ pc}$ ($= 3 \times 10^{15} \text{ cm}$). Thus an unstable wave may grow considerably before it convects out of the beam channel.

Because of the two-stream instability, plasma waves of finite but small amplitude are expected to coexist with the plasma in the beam channel^{24,25}. One important effect of the waves is to produce an anomalous electrical resistivity (which does not depend on binary collisions). This resistivity acts to prevent electrons from partially shorting the proton accelerating potential. A self-consistent, non-linear theory of the interaction would determine the neutralisation length z_{neut} . It is of course possible that the nonlinear theory would allow only a quasi-periodic acceleration²¹ of protons with a time scale $\sim z_{\text{neut}}/c$.

Linear instability of the proton beams may also involve the circularly polarised Alfvén and/or cyclotron plasma waves propagating parallel to the magnetic field. The interaction of streaming cosmic rays with Alfvén waves has been studied^{26,27}, but apparently only under conditions where the cosmic rays are nearly isotropic.

For distances $|z| > z_{\text{neut}}$, the magnetic field is the 'stretched-out' poloidal field of the accretion disk, and is nearly parallel to the local proton velocity. For example, if the magnetic flux through the disk is $10^{23} \text{ gauss cm}^2$ (from equation (3)) then

$$B_z \sim 10^{-4} \left[\frac{pc}{a(z)} \right]^2 \text{ gauss} \quad (12)$$

where $a(z)$ is the beam radius. The proton gyro-radii implied by equation (12) are $\simeq 10^2 \text{ pc} \sin(\theta)$ ($\gamma/10^{10})(a/pc)^2$, where $\theta \ll 1$ is the pitch angle.

The electron beams

It is possible that the energy loss per unit distance of the proton beam is determined by the two-stream interaction. A small fraction of the cold electrons in the beam channel get trapped and untrapped in the unstable plasma waves and are thereby accelerated to relativistic energies^{24,25}. At the same time the proton spectrum is shifted to lower energies. A nonlinear theory is required to predict the energy spectra of the electrons and protons. It is, however, relevant that equation (11) implies that an electron trapped with the phase velocity of an unstable plasma wave has a Lorentz factor

$$\gamma_w = \Gamma(\psi^{2/3} + \Gamma^2 \psi^2)^{-1/2}$$

$$\Gamma \equiv 2^{1/6} \left(\frac{\omega_p}{\omega_B} \right)^{1/3} \sim 5 \times 10^3 \left(\frac{n_p}{10^{-3} \text{ cm}^{-3}} \right)^{1/6} \left(\frac{\gamma}{10^{10}} \right)^{1/6} \left(\frac{a}{pc} \right)^{1/3} \quad (13)$$

where we have taken $n_B = 10^{-12} (pc/a)^2 \text{ cm}^{-3}$. The energetic electrons are collimated with the proton beams, having small pitch angles and small synchrotron losses. An upper limit on the range of electron Lorentz factors may however occur because the pitch angles are not negligibly small. For small pitch angles the synchrotron radiation is collimated with the proton beams.

The radio components

The collimated propagation of the oppositely travelling beams of electrons terminates in two regions of size a_R at $z = \pm z_R$, which are the radio components. In these regions the magnetic field of the beam channel connects with a non-uniform irregular field. Thus relativistic electrons arriving at $\pm z_R$ rapidly lose their energy by incoherent synchrotron emission. The collimated propagation of protons with gyro-radii $\gg a_R$ continues beyond z_R , and these protons may continue to accelerate cold electrons by the two-stream interaction. The observed values of z_R extend from $< 1 \text{ pc}$ to $\sim 1 \text{ Mpc}$. Consider first the small sources with, say, $z_R < 10^2 \text{ pc}$. The magnetic field is that carried by the beam (equation (12)). The momentum of the beam electrons corresponds to a pressure $\lesssim P_R \equiv (L_R/\pi c) [a(z)]^{-2}$, where L_R is given by equation (5). The electron pressure compresses the external medium and gives rise to shock wave structures^{28,1} at $z = \pm z_R$. These structures may move with relativistic velocities in the $\pm z$ directions if $P_R > n_{\text{ext}} m_p c^2$, or $L_R > 10^{42} (a/pc)^2 (n_{\text{ext}}/10^{-3} \text{ cm}^{-3}) \text{ erg s}^{-1}$, where n_{ext} is the ambient density of the external medium. For the extended sources with $z_R > 10^2 \text{ pc}$, the location of the shock structures (and associated radio emitting regions) is determined by the pressure balance conditions^{28,1} $P_R \gtrsim n_{\text{ext}} m_p U_z^2$, where U_z ($< c$) is the velocity of the structures in the $\pm z$ directions into the external medium. For the large sources, the magnetic field carried by the beam is exceedingly weak. Amplification of the field may, however, occur by field line stretching at the expense of the energy of the turbulent motions of the plasma and relativistic electrons. Subsequent to the formation of an extended double source, a second pair of oppositely travelling shock wave structures may be generated, for example, by an instability involving the accretion disk. The secondary shock structures will be aligned with the disk angular momentum and thus approximately aligned with the extended double.

The high energy protons which reach the large radio components escape into extragalactic space without appreciable synchrotron losses if the magnetic field strengths are $< 10^{-3} \text{ gauss}$. It appears possible that the accumulation over time of the high energy protons lost from nearby ($\lesssim 300 \text{ Mpc}$) dynamo sources has produced the high-energy end (10^{16} – 10^{20} eV) of the observed cosmic ray spectrum.

An analytic solution to the electrodynamics of a magnetised accretion disk has recently come to our attention²⁹.

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Actual and anticipated petrographic effects of carbonate undersaturation in shallow seawater

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In the near future, atmospheric carbon dioxide from the combustion of fossil fuels may cause the surface layers of the ocean to become generally undersaturated with calcium carbonate. The hypothetical sedimentary effects of such an event are here discussed in the light of studies in the Skagerrak Bay, North Sea. In this area, nearshore sediments in the depth range 0–40 m are affected by present-day carbonate dissolution. Not surprisingly, evidence of inorganic carbonate precipitation is absent. Stages in the dissolution process are microscopic etch patterns on calcareous grains, partially dissolved grains, and finally structural breakdown and complete dissolution of skeletal elements. Mineral phases affected are calcite, aragonite, and a spectrum of magnesian calcites. Dissolution is quantitatively unimportant in living calcareous organisms, but preservation of shells and skeletons as fossils is much impeded. A future change towards similar conditions in all the world's shelf seas would certainly mean an end to the typical warm-sea carbonate sedimentation as it is known today on the Bahama Banks and in other supersaturated regions.

It is a serious possibility that the industrial production of atmospheric carbon dioxide will cause the surface layers of the sea to become generally undersaturated with calcium carbonate—perhaps within no more than 30 yr (refs 1–6). If indeed this prospect is true—the hypothesis has its opponents⁷—it will have radical consequences for carbonate sedimentation in the world's shelf seas which are now mainly supersaturated. I attempt to show here that the diagenetic processes affecting carbonate sediments in undersaturated waters are in striking contrast to what is known from warm, carbonate-supersaturated regions. Should carbonate diagenesis of the undersaturation model indeed become universal in the world's shallow seas by the turn of the century, the event would certainly mean an end to the typical warm-sea carbonate sedimentation as we know it today. Carbonate sedimentation in cold, high-latitude seas would become still more restricted. A change of this nature would leave an extensive mark of lasting geological significance in the shelf sediments, well worthy of its own footnote in the history of man's influence on the natural environment.

I can take no stand for or against the probability of a future carbonate undersaturation in the surface layers of the sea. Others are conducting that debate^{1–7}. My intention is to point out some of the effects such an event might have on existing carbonate sediments and related organisms in shallow waters—if ever it does occur.

My story is based on factual observations in the present-

day sea. Contrary to common belief, areas with carbonate-undersaturated shallow marine waters actually exist at the present time, and they seem to be the result of natural—as contrasted to man-made—environmental conditions. Such is the case, for example, in the Skagerrak Bay in the North Sea, where the petrographic effects of carbonate undersaturation are very prominent in nearshore sediments in the depth interval 0–40 m (refs 8–10).

Knowledge in this particular field of marine carbonate sedimentation is still fragmentary, and this account can touch only on two basic aspects of the problem: first, the evidence of marine carbonate undersaturation, and second, the most obvious effects of carbonate dissolution in nearshore sediments and related organisms.

Petrographic saturometry

No one really knows the precise level of carbonate saturation in the sea, and estimates vary considerably, both of the undersaturation in the deep sea^{11–13}, and of the supersaturation in surface waters^{14–16}. The disagreement on this point need not concern us here, however, since undersaturation in natural sedimentary environments can be diagnosed by a direct petrographic approach. The most explicit evidence of marine carbonate undersaturation is obviously the condition that carbonate sediments dissolve. During the Challenger expedition—which ended on May 24 exactly 100 yr ago—the realisation that foraminiferal ooze dissolves at depth meant the original discovery of marine carbonate undersaturation (ref. 17). Today, dissolution can be petrographically diagnosed in exceedingly small samples, even in individual sediment grains, by means of scanning electron microscopy (SEM), and one is motivated to speak of petrographic saturometry. The method is sensitive only to such levels of undersaturation that actually lead to dissolution, and hence is well suited for field studies of carbonate diagenesis. The primary diagnostic criteria are microscopic etch patterns which develop on the surfaces of sediment grains as soon as these are affected by dissolution⁹. Each kind of calcareous grain—a mollusc shell, a barnacle plate, an echinoid stereome—has its own distinctive etch pattern which is related to the mineralogy and structure of the grain fabric (Fig. 1). The SEM analyses allow identification of separate mineral species in the grains, and the degree of undersaturation thus can be related to the solubility of the various minerals, and even to different phases of the same mineral^{8–10}.

Petrographic saturometry in natural sediments has its limitations, however. Most important here is that etch patterns may either reflect uninterrupted dissolution in continual undersaturation, or they may be cumulative records of several dissolution events, representing occasional drops of the saturation level in an otherwise saturated, or even supersaturated, environment.

In the Skagerrak—as in other high-latitude shelf seas—seasonal fluctuations in carbonate saturation seem very likely. Three factors contribute to lower saturation levels

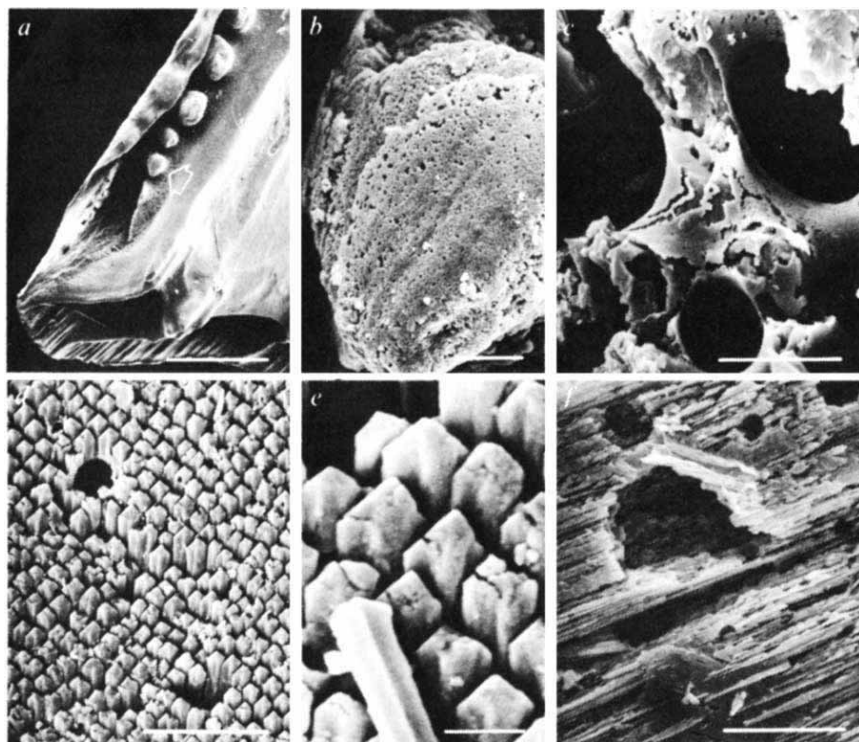


Fig. 1 Dissolution features in carbonate sediments from the Skagerrak: SEM micrographs, scale bars 10 μm , except for (a) 250 μm , and (e) 2 μm . *a*, Plate of dead barnacle collected *in situ* on rock face at 4-m depth. The entire shell was weak and crumbly. Arrow points to hinge tooth shown in detail. *b*, Corroded appearance of the calcite fabric in barnacle hinge tooth. *c*, Echinoderm fragment of magnesian calcite, grain size fraction 125–250 μm , water depth 12 m. Echinoderm fragments behave as single calcite crystals in the petrographic light microscope, but reveal an intricate core-and-rind structure during natural dissolution. *d*, Etch pattern exposing the tips of prismatic calcite fibres in *Mytilus* fragment. Grain size fraction 250–500 μm , water depth 12 m. *e*, Tips of etched *Mytilus* fibres. *f*, Transverse fracture through cross-lamellar aragonite in bivalve shell, showing partial dissolution of the fabric. The holes are corroded borings after endolithic organisms. Grain size 4 mm, water depth 6 m.

during the winters: first, photosynthesis is at its minimum so organic uptake of CO_2 is small; second, water temperatures are low so solubility of CO_2 is high and third, large amounts of CO_2 are released in the water through the decay of the summer crop of organisms.

Carbonate diagenesis in the Skagerrak

Even a cursory examination of the processes in the Skagerrak will show the fundamental differences between the carbonate-supersaturation diagenetic model so well known from warm shallow seas (refs 18, 19), and the undersaturation model in these cooler waters. The effects of a universal change to carbonate-undersaturated conditions may then be more properly assessed.

In the depth interval studied (0–40 m), the carbonates derive mainly from macroscopic benthic organisms, such as, bivalves, gastropods, barnacles, echinoderms and calcareous algae. Benthic foraminifers are of local importance. The most common single constituent is the blue mussel *Mytilus edulis* whose shell fragments give many sands a bluish tinge.

The sediments studied are nearshore sands of varying carbonate content, from a few % by weight in terrigenous sands, to > 90% by weight in skeletal sands. The grain size ranges from several cm in complete shells, to a few μm in the most comminuted material, with the median value commonly in the 200–800- μm interval.

From a mineralogical point of view, the carbonate fraction consists of aragonite, calcite, and a spectrum of magnesian calcites (Fig. 2). The proportions of mineral species in the sediments depend on the composition of the local carbonate-producing population, but calcite is nearly always dominant.

The effects of carbonate dissolution in the sediments can be seen with a simple binocular microscope: calcareous grains have a frosted, sometimes even chalky appearance, and many grains are weak and crumbly. The frosted texture is caused by surface etch patterns, whereas the chalky and powdery state originates from partial dissolution of the grain fabric.

In the Skagerrak, etch patterns occur on all kinds of

calcareous grains, and it is clear that the environment is undersaturated with respect to all carbonate phases present: calcite, aragonite, and magnesian calcites^{8–10}. In principle, the solubility of the carbonates varies according to the following pattern: most soluble among the sediment constituents are magnesian calcites with ≥ 15 mol % MgCO_3 in solid solution; next come aragonite and the intermediate magnesian calcites, whereas pure calcite is the least soluble^{20–22}. It is, however, a complicating factor that biogenic carbonate grains are intricate constructions of organic matrices and mineral substance, with the mineral part organised in various submicroscopic units of calcification. Within a single grain, the skeletal units may vary considerably in solubility because of variations in mineralogy and composition^{23–25}.

The heterogeneous nature of the skeletal grains determines their dissolution behaviour. Minor spatial variations in solubility in the particle fabrics control the etch topography which develops as soon as dissolution begins (Fig. 1). On continued exposure, deeper parts of the grains are gradually affected and the intragranular fabrics are partially dissolved. The process produces the familiar chalky appearance of many skeletal grains⁸. An instructive example is the cross-lamellar aragonite fabric in mollusc shells: some lamellae are selectively dissolved out of the shells while other lamellae remain as a residual chalky fabric (Fig. 1f).

This mechanism suggests that the rate of dissolution is slow so that the ambient undersaturation is not fully compensated by dissolution at the grain surface but exists as an undersaturation gradient some distance into the grain. The depth of the zone thus affected by intragranular dissolution is several hundred μm , and skeletal grains of medium sand size and finer may be affected by dissolution right through the particle fabric.

An interesting aspect of the degradation of skeletal carbonates in the Skagerrak is the decomposition of intraskeletal organic substances. The decomposition contributes heavily to the structural breakdown of skeletal grains, and the process is particularly evident in mollusc shells. In these, decomposition affects, for example, the organic sheets between layers of aragonite nacre and the

sheaths around the calcite fibres in *Mytilus* shells. The aragonite nacre peels off in thin flakes, whereas the prismatic calcite disaggregates to free fibres, and both components last on their own for some time. Aragonite flakes and *Mytilus* fibres are the dominating constituents in a subordinate 'lime mud' fraction which is the final stage before complete dissolution (Fig. 3). A similar disaggregation of mollusc shells or other animal carbonates is not known from warm, carbonate-supersaturated waters, but why this is so is not understood—particularly not in the light of the abundant evidence of disaggregation of calcareous plant tissue. Recent studies indicate that the disaggregation of calcareous algae is the main producer of lime muds in warm shallow environments²⁶.

The Skagerrak sediments differ from warm-sea carbonate sands, not only by having a specific set of dissolution features which are unknown in carbonate-supersaturated areas, but also by lacking the various signs of precipitation which accompany supersaturation. The relationship between supersaturation and precipitation in seawater is in itself a complex problem^{22,27}, and it seems that the ordinary carbonate supersaturation in warm surface waters leaves a rather erratic petrographic record in the sediments^{10,18,19}. Sand constituents commonly attributed to precipitation are ooids, grapestones, cemented pellets, and cements in micrite envelopes and hollow grains^{18,19,28,29}. As might be expected, such constituents do not occur in the Skagerrak carbonates.

The only carbonate cements observed are all small amounts of internal aragonite and Mg-calcite cement, formed within coralline algal nodules and crusts through the calcifying processes of the red algae³⁰. This biogenic cement is totally dependent on the algal life processes, and after death of the algae both skeletons and cement are attacked by dissolution.

The picture emerging from the petrographic studies of carbonate diagenesis in the Skagerrak is very consistent. It

Fig. 2 X-ray powder diffractograms of various size fractions in a calcareous sand taken with Ni-filtered Cu-radiation. The black peaks at 26 and 27 are aragonite, the broad peak at 29–30 is calcite with the Mg-calcite area hatched. Relative amounts of the minerals can be calculated from peak areas (ref. 19). The relative amounts of Mg-calcite and aragonite increase with decreasing grain size, indicating a more rapid structural breakdown of these constituents.

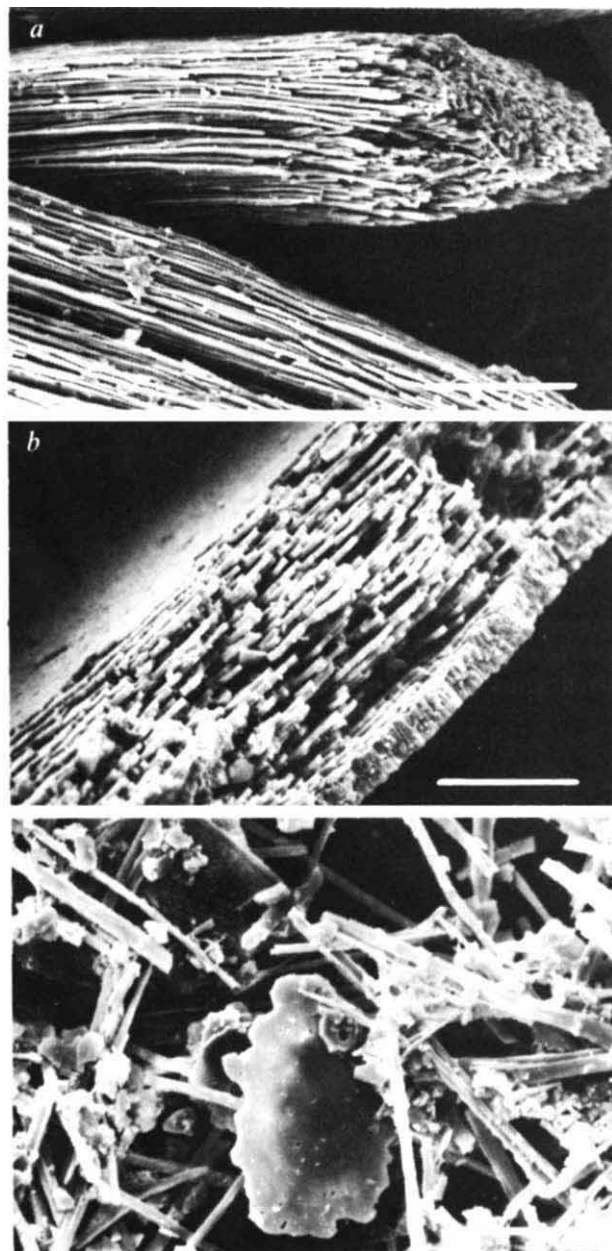
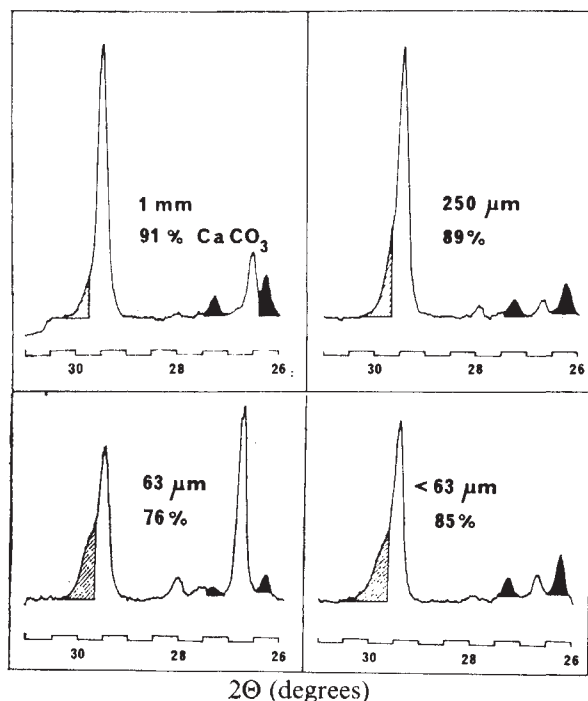


Fig. 3 Structural breakdown of skeletal carbonates in the Skagerrak: SEM micrographs, scale bars in (a) 50 μm , in (b, c) 20 μm . a, *Mytilus* fragments in their natural state. The calcite part of the shells is reduced to bundles of weakly coherent elastic fibres which easily disaggregate to free fibres. Grain size fraction 125–250 μm , water depth 12 m. b, Transverse fracture through bivalve shell with layers of peeling aragonite nacre. Grain size 1 mm, water depth 6 m. c, Molluscan aragonite flakes and *Mytilus* fibres in a 'lime mud' fraction of skeletal debris. Grain size fraction < 63 μm , water depth 15 m.

shows the absence of carbonate precipitation related to ambient supersaturation, and instead the presence of carbonate dissolution because of ambient undersaturation.

Is the dissolution really caused by ambient carbonate undersaturation in the seawater? Considering the various processes of organic decay that must occur in the sediments—especially when the summer crop of organisms dies off in response to the harsh winter conditions—is it not more likely that dissolution is an interstitial process at some depth in the sediments? This question was studied by monitoring the breakdown of calcareous epibionts on submarine rock surfaces away from the sediments, collecting skeletal material during SCUBA dives every fourth month. The results show that dead epibionts, such as coralline algal crusts, barnacle cups, and serpulid tubes, are attacked by

dissolution while still in growth positions on naked rock-faces. The barnacle plate in Fig. 1 is an example of this. To a diver, accustomed to cuts and scratches from barnacles on rocky shores, it was a surprise sometimes to find as much as one third of a barnacle colony as friable cups which could be crushed with the tip of a finger.

The impression is that isolated calcareous organisms, freely exposed to circulating seawater on stones and rocks, are more rapidly destroyed than organisms and carbonate grains within calcareous sands. Dead calcareous epibionts disappear from their growth sites in a year or two, partly because of decomposition of the binding organic substances, and partly because of carbonate dissolution. For example, *Mytilus* shells fall off and go into the sediments because of organic decomposition, as do barnacle cups with the exception of the basal plates. Barnacle basal plates, crustose coralline algae, and serpulid worm tubes are firmly cemented to the rocks and provide good examples of dissolution *in situ*. It is worth noting that calcareous crusts do not accumulate on rock surfaces in the area, even if growth of encrusters is vigorous—a marked contrast to the conditions in many warm, supersaturated seas.

In living organisms, susceptibility to carbonate dissolution varies widely among different groups. The decisive factor is the degree of organic protection of the mineral phase, and this depends on the structural organisation of the organism. In living crustose coralline algae, echinoderms, and benthic foraminifers, the skeletons are fully protected by living substance and there is no dissolution. As previously mentioned, crustose coralline algae actually control the microenvironment in their crusts to the extent that intraskeletal carbonate cement may form in spite of the ambient undersaturation³⁰. More exposed to dissolution are the shells of bivalves, gastropods, and barnacles, and etch patterns have been observed, for example, on the apical ends of shells of living gastropods. Quantitatively, however, the dissolution is insignificant in these large animals, and their life processes are unaffected. The conditions may be different for microscopic organisms suspended in seawater—for example coccolithic algae—but calcareous plankton has not yet been studied from this point of view.

As constituents of calcareous sands, skeletal fragments obviously may last for a very long time. In areas of net accumulation of sediments, the grains are buried by sedimentation and are then out of reach of the marine carbonate dissolution which does not proceed far beneath the sediment-seawater interface. The destiny of an individual calcareous sediment grain depends on a complex set of processes, involving organic carbonate production, physical sedimentation processes, wave and current action, bioturbation, bioerosion, and the degree of ambient carbonate saturation. How to define a meaningful 'rate of dissolution' in these conditions, and how to calculate such a parameter, are problems which remain to be solved.

Concluding remarks

As mentioned earlier, knowledge of carbonate undersaturation in shallow seawater is still fragmentary, and the story from the Skagerrak must be left with several holes in it. Perhaps the most annoying question is: why is carbonate undersaturation in shallow seawater so little understood from a chemical point of view? To my knowledge, the consensus among chemists is that the surface layers of the sea are supersaturated throughout, although there are discussions as to the degree^{6,14-16}. Part of the answer may lie in a sampling problem. Few, if any, saturation data represent the nearshore conditions in the Skagerrak, let's say in the dark and cold at four o'clock on a January morning, when the sublittoral environment is absolutely devoid of photosynthesis, but has considerable respiration and bacterial decay. Should carbonate undersaturation occur in

these conditions, however, it would be faithfully recorded as dissolution features in the sediments, clearly diagnostic in petrographic analysis.

In the absence of chemical data, it cannot be determined whether carbonate undersaturation in the Skagerrak is general and permanent, or if it is limited in space and time—for example, to the bottom waters in winter. Even if we assume that undersaturation here is limited, it does not, however, invalidate the main hypothesis of this paper—namely, that a future change to general carbonate undersaturation in the ocean's surface layers would cause radical changes in carbonate sedimentation in the shelf seas. Rather on the contrary: if an extensive dissolution record like the one in the Skagerrak can be produced by fairly limited carbonate undersaturation, how severe must be the effects of a universal change.

As far as it goes, the record in the Skagerrak sediments is entirely consistent. First, there is no petrographic evidence of ambient carbonate supersaturation, as known from warm shallow seas. Second, all carbonate phases present—calcite, aragonite, and magnesian calcites—are attacked by dissolution and gradually lost from the sediments. The petrographic record of the dissolution process consists of etch patterns and partially dissolved grains. The structural breakdown of skeletal carbonates is much promoted by decomposition of intraskeletal organic substances.

A change towards similar conditions in all the world's shelf seas would mean a radical change of the present pattern of carbonate sedimentation. The changes cannot be calculated in detail, especially the net effects of dissolution. From a petrographic point of view, an early and fundamental effect would be the termination of shallow marine carbonate precipitation. This would mean an end to largely inorganic processes such as growth of ooids, and formation of cements in grapestones, micrite envelopes, pellets, hollow grains and other sand constituents: probably also to interparticle cementation in submarine sands and beachrock. The pattern of preservation of calcareous shells and skeletons would certainly change, with effects on the accumulation of fossil assemblages in the sediments.

Sedimentary changes of this nature would stand out most drastically in present areas of high carbonate supersaturation. They would mean an end to—or rather an interruption of—the typical warm-sea carbonate sedimentation as we know it from, for example, the Bahama Banks and the Persian Gulf. In geological terms, a man-made carbonate undersaturation would probably be a brief event, but it would be irrevocably recorded in the sediments and thus take its place in the general geological record with its amazing permanence. Every geologist who has studied, for example, the details of an Ordovician limestone will probably share my conviction that sedimentary changes, however subtle, in carbonate sediments have the potential to last for hundreds of millions of years. An event of man-made carbonate undersaturation in the shallow seas might indeed produce one of the most universal and permanent records of human activity ever formed on Earth.

In a shorter perspective, it is reassuring that the evidence from the Skagerrak of today indicates that carbonate undersaturation in shelf seas does not necessarily mean serious problems for the marine environment and its inhabitants.

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Cellular basis for the T wave of the electrocardiogram

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Differences in action potential duration in different regions of the mammalian ventricle are not systematically present when quiescent tissue is first stimulated, but develop rapidly during repetitive activity. The effects of ouabain and temperature suggest the involvement of the $\text{Na}^+\text{-K}^+$ exchange pump.

THE electrocardiogram (ECG) has proved to be a remarkably useful tool in the diagnosis and management of many types of malfunction or disease of the heart. But after nearly a century of use and refinement, and in spite of recent advances in cellular cardiac electrophysiology, certain features of the ECG are not well understood. One example is the T wave of the mammalian ECG. It is empirically described as arising from differences in action potential duration in different anatomical regions of the ventricle. Most attempts to account for the T wave have utilised indirect measurements of the sequence of repolarisation in ventricular tissue. For example, detailed studies of the relative refractory period in several anatomical locations in both ventricles and on the intraventricular septum have been reported¹. And Spach and Barr² have used arrays of chronically implanted electrodes simultaneously to record potential distributions in the intact dog heart during excitation and repolarisation. We have used conventional micro-electrode techniques to record action potentials in isolated preparations from sheep ventricle. The results show unexpected, activity-dependent changes in action potential duration which may provide further understanding of the mechanism of the T wave.

The T wave of the mammalian ECG is synchronous with the repolarisation phase of the ventricular action potential, and is therefore thought to represent the net extracellular field produced by the spreading of the repolarisation wave throughout the ventricular myocardium. But it is somewhat surprising that the T wave is found to be positive in the same ECG leads as the R wave of the QRS complex, since this complex corresponds to depolarisation of the ventricular myocardium.

Extracellular recordings of the potentials produced by the conduction of nerve impulses produce a biphasic wave, so that, if the leads are arranged such that depolarisation corresponds to a positive extracellular wave, repolarisation is represented by a negative extracellular wave. This result is necessary if depolarisation and repolarisation propagate in the same direction.

The apparent paradox in the directionality of the T wave

is usually explained by postulating that the depolarisation and repolarisation waves in at least some parts of the ventricle travel in opposite directions, so that regions that are excited latest repolarise earliest. This explanation requires that the duration of the action potential should be greater at the point of entry of the wave (in the endocardial muscle of the septum) than at later points in the conduction pathway (for example, in the epicardial surface of the apex). Early work using extracellular electrodes provided strong evidence for this conclusion (for discussion and references see ref. 3). More recently, recordings made with intracellular microelectrodes have shown that, during normal repetitive activity, the action potential at the epicardial surface of the ventricle is shorter than that at the endocardial surface⁴.

An important possible function for such a difference is that the longer action potential at early points in the conduction pathway may prevent re-excitation and re-entry. It is therefore important to attempt to determine the cellular basis for this difference. The experiments we describe here show two important results. First, the duration differences responsible for the normal T wave develop as a consequence of repetitive activity; they are not present in the first action potential of a previously quiescent ventricle. Second, the effects of repetitive activity are strongly influenced by cardiac glycosides and by temperature.

Small pieces of tissue (2-4 mm square and 1 mm thick) were dissected from two regions of sheep left ventricles—the upper part (base) of the interventricular septum and the epicardial surface of the apex. The former is endocardial and the latter is epicardial. Some theories of the T wave regard the difference in action potential duration as being primarily between endocardial and epicardial surfaces. The alternative view is that a base/apex gradient exists. The two views are not, of course, mutually exclusive. Our choice of regions allows the correlate of either kind of gradient to be detected in action potential recordings. For simplicity we refer to the two regions as 'base' and 'apex', respectively.

After a suitable period for recovery (20-30 min) action potentials from each region were recorded using conventional 3-M KCl microelectrodes. All measurements of action potential duration were made from heat-sensitive pen recordings at the point of 80% repolarisation. In contrast to recordings made on the whole heart, it is possible to remain impaled in small pieces of tissue for prolonged periods. Moreover, since the preparations are quiescent until

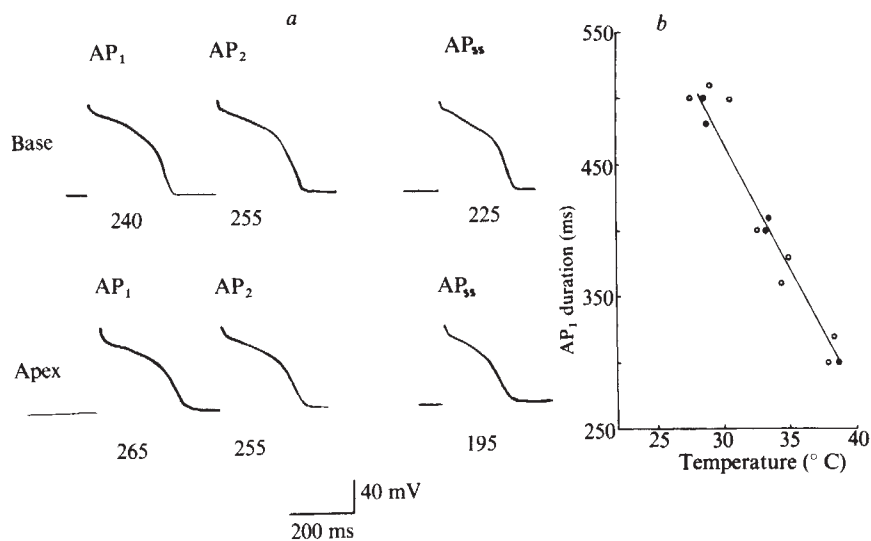


Fig. 1 *a*, Records obtained during trains of action potentials produced in base (endocardial) and apex (epicardial) preparations from sheep ventricle. Stimuli were applied at 150 per s after a 30–60-s quiescent period. The time to 80% repolarisation is shown in ms below each action potential. AP₁ is the first action potential in each train, AP₂ the second and AP_{ss} is taken when there are no further changes (usually 10–20 action potentials). There is a considerable difference in the effect of activity in the two regions. At the base AP₂ lengthens and AP_{ss} is only 15 ms shorter than AP₁. At the apex AP₂ shortens and AP_{ss} is 70 ms shorter than AP₁. These particular records show a 25-ms difference between AP₁ in the two regions. This difference is in the wrong direction for explaining the normal T wave and was not found to be significant (see *b*). *b*, Duration of AP₁ as a function of temperature. There is no systematic difference between AP₁ durations at the base (●) and apex (○). Similar results were found in other experiments (although the precise functional dependence of duration on temperature was not always linear).

stimulated it is also possible to determine whether any differences that occur in the action potentials are dependent on the presence of repetitive activity.

First action potential

This question was answered by studying trains of action potentials following a short (30–60 s) period of rest. We found that there were no systematic differences between the first action potentials in the base and apex regions. Moreover, this similarity between the two regions occurred at all the temperatures investigated. Figure 1*b* shows a plot of the first action potential durations obtained from the base (filled circles) and apex (open circles) between 28 and 38 °C. There is a scatter of up to about 20 ms in the durations but it is clear that the durations are not systematically different. They can be fitted by the same relationship as a function of temperature. This result was also obtained in other experiments provided that adequate time was allowed for recovery and that the results were compared from pieces of tissue taken from the same heart.

These experiments strongly suggest that there are no substantial differences between the basic ionic conductance mechanisms that control the plateau and repolarisation phases in the two regions. If such differences exist we should have to suppose both that differences in the individual conductance mechanisms are balanced to produce identical durations and that this balance is preserved for the whole temperature range. This seems very unlikely in view of the fact that the net current flow during the plateau is approximately 1% of the magnitude of the individual inward and outward currents involved⁵.

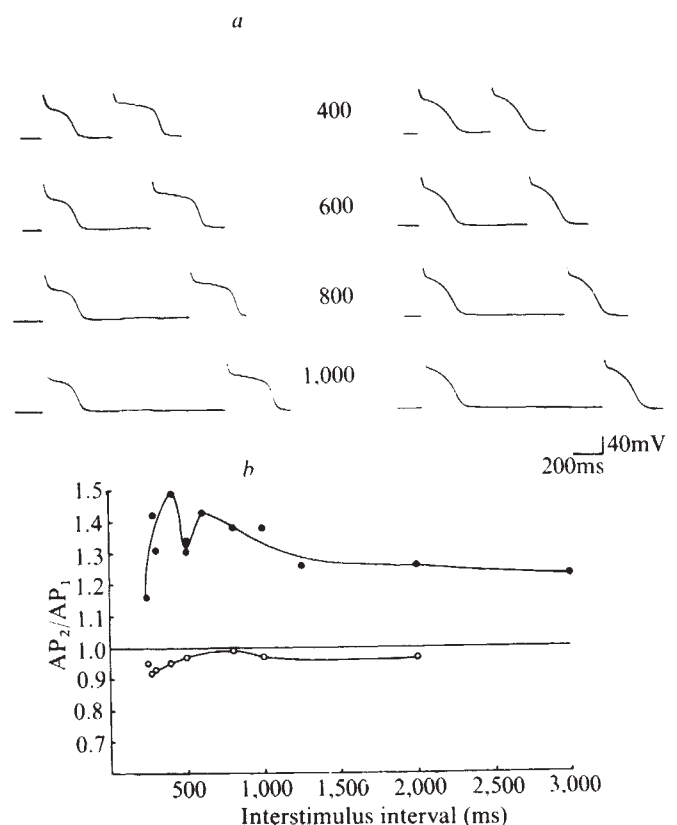
Repetitive activity and second action potential

Although there are no systematic differences between the first action potential durations, it is clear from Fig. 1*a* that the effect of activity is strikingly different in the two regions. At the base we invariably found that the second action potential duration (AP₂) is substantially longer than the first (AP₁) and that the steady-state action potential (AP_{ss}) is either similar to or only slightly shorter than the first. In the case illustrated, AP_{ss} is 225 ms compared with 240 ms for AP₁. In contrast, at the apex we generally found a substantial shortening so that AP_{ss} is much shorter than AP₁. This effect is particularly striking in Fig. 1*a* since the duration of AP₁ at the apex (265 ms) is longer than at the base (240 ms). Such a difference would result in a negative T wave, as would also be the case when the durations are identical. Nevertheless, in the apex AP_{ss} is 70 ms shorter than AP₁ and becomes 30 ms shorter than AP_{ss} at the base.

Such a difference would lead to the positive T wave observed during normal repetitive activity. We shall discuss the results for AP₂ and AP_{ss} separately.

Twin pulse studies on sheep Purkinje fibres usually show that the second action potential shortens as the interstimulus interval is reduced. This shortening is primarily caused by residual activation of i_{K1} , the outward current which speeds repolarisation⁶. But lengthening of the second action potential has also occasionally been observed in Purkinje fibres⁷. Such experiments have shown that 10⁻⁶ M ouabain reduces or eliminates the increase in duration of AP₂.

Fig. 2 *a*, Records obtained during paired pulse stimulation at various intervals (indicated in ms). *b*, Duration ratios (AP₂/AP₁) as a function of interstimulus intervals from experiment illustrated in (*a*). The solid curves were drawn by eye. Note that the ratio is less than 1 at the apex (○) and substantially greater than 1 at the base (●).



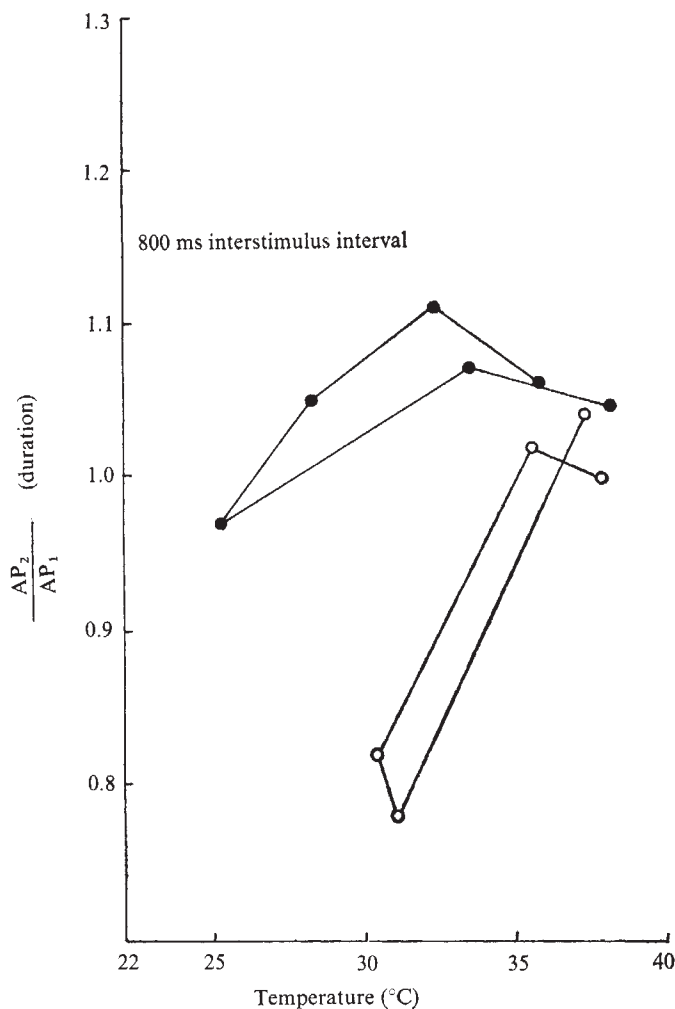


Fig. 3 Influence of temperature on the AP_2/AP_1 duration ratios at the base (●) and apex (○) at an interstimulus interval of 800 ms. Cooling has a larger influence on the apex than on the base. The effects are completely reversible in both regions.

We have studied the duration of AP_2 after application of paired stimuli at various interstimulus intervals to the base and apex regions of the ventricle. As before, a 30–60-s rest period followed each pair of stimuli. As shown in Fig. 2a, AP_2 at the base was substantially longer than AP_1 , whereas the opposite result was obtained at the apex. Figure 2b shows plots of the AP_2/AP_1 ratio as a function of interstimulus interval. At all intervals, the ratio is much smaller at the apex than at the base. This result was obtained in all healthy preparations. It was important, however, to allow adequate time (about 1 h) for recovery after dissection. During recovery in apex preparations, while the resting potential was low and the first action potential was relatively short, a striking prolongation of AP_2 could be obtained. Such preparations conformed to the pattern of response shown in Fig. 2 after they reached their normal resting potential and AP_1 became normal in duration.

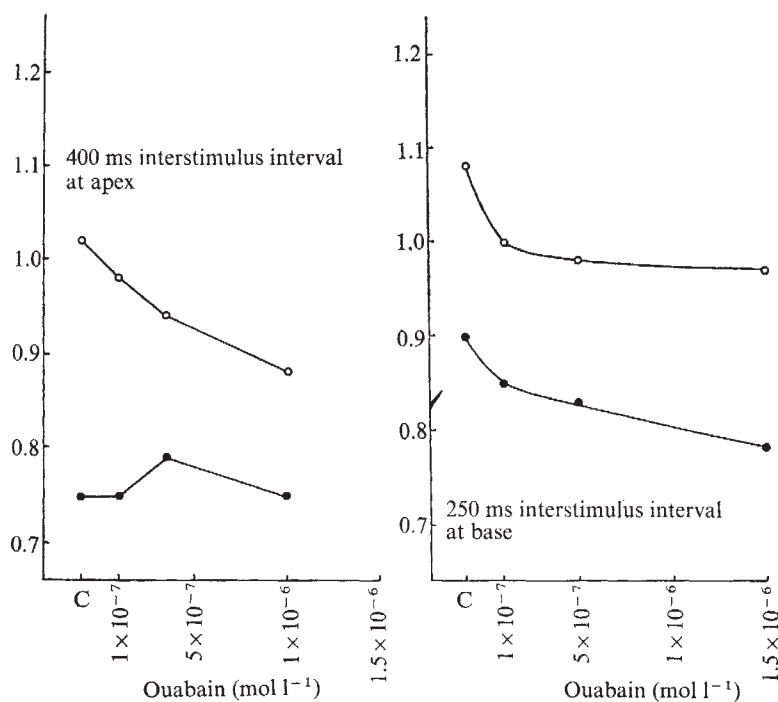
To test whether the prolongation of AP_2 at the base is attributable to the activity of the Na^+-K^+ exchange pump, we used two methods of blocking pump activity—cooling and application of ouabain. Figure 3 shows the results of cooling at an interstimulus interval of 800 ms. In both regions the AP_2/AP_1 duration ratio becomes smaller at low temperatures but the effect was always found to be more dramatic at the apex than at the base. At 38 °C the AP_2/AP_1 ratio is slightly larger than 1.0 in the apex but falls to about 0.8 when the tissue is cooled to 30 °C. By contrast, in the base, the ratio is still larger than 1.0 at that temperature and cooling to 25 °C was required to reduce the ratio significantly. These effects were totally reversible when the preparations were rewarmed.

Figure 4 shows the results of experiments on the effects of various concentrations of ouabain. In both regions, the AP_2/AP_1 ratio is significantly reduced by ouabain at concentrations that are thought to reduce the activity of the pump significantly. Unlike cooling, these effects were not reversible. This result is not surprising in view of the well known difficulty in reversing the inhibitory action of ouabain on the $Na-K$ exchange pump.

Steady-state action potential

It is clear from the results described so far that very marked differences between the base and apex regions may develop

Fig. 4 The effects of ouabain on AP_2/AP_1 (○) and AP_{ss}/AP_1 (●) duration ratios. The stimulus frequency is 240 per s at the base and 150 per s at the apex. Further explanation in text.



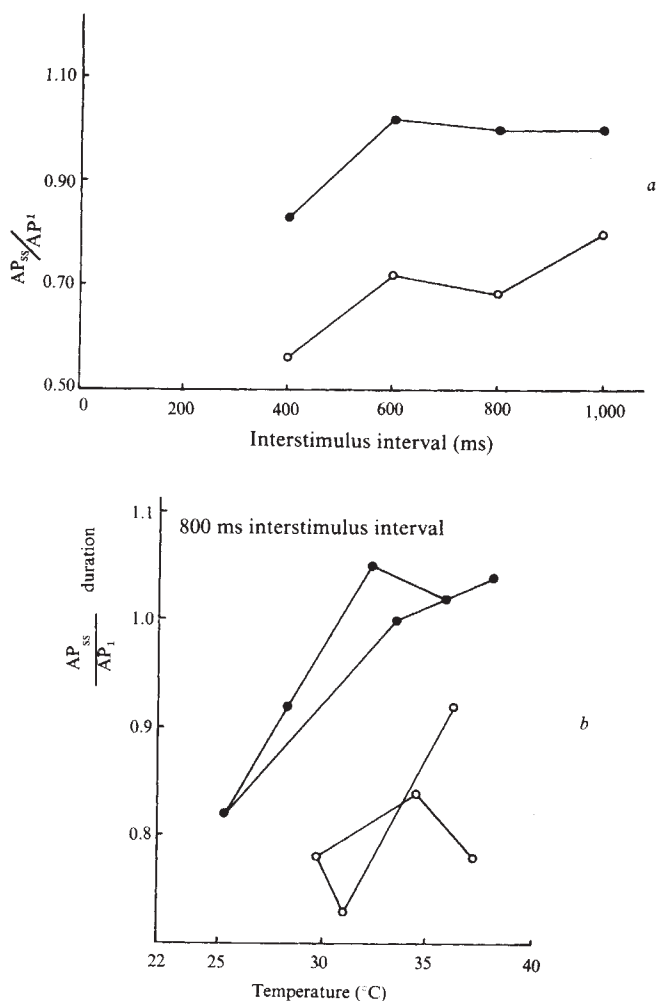


Fig. 5 *a*, AP_{ss}/AP_1 duration ratios at base (●, 38.6 $^{\circ}C$) and apex (○, 38.0 $^{\circ}C$) as a function of interstimulus interval. The precise influence of changes in frequency on AP_{ss} in the two regions varies somewhat between experiments but, as in this experiment, a significant difference between the two areas is found over the whole frequency range investigated. *b*, Influence of temperature on the AP_{ss}/AP_1 duration ratios in the base (●) and apex (○). These results can be compared with those for the AP_2/AP_1 ratios shown in Fig. 3. The same general phenomenon is observed, that is the apex ratio is reduced to lower levels than the base ratio by cooling. In this particular experiment, the effects were perfectly reversible at the base but not at the apex. Reversible effects on AP_{ss} were obtained in the apex in other experiments.

as a consequence of only one action potential. The difference between the durations of AP_2 in the two regions would produce a positive T wave. It is important, however, to remember that the normal T wave is determined by the steady-state action potential characteristics of maintained repetitive firing. It is therefore essential to study the durations of AP_{ss} in each region of the ventricle.

Figure 1*a* shows that AP_{ss} is longer at the base than at the apex. Figure 5*a* shows that this difference persists for a wide range of frequencies. The filled circles show the AP_{ss}/AP_1 ratio at the base as a function of interstimulus interval. The results for the apex are shown by the open circles. Although in both cases there is a tendency for the ratio to decrease at high frequencies, the difference is always substantial. This result suggests that changes in frequency *per se* should not influence the polarity of the T wave.

Figure 5*b* shows the influence of cooling on the AP_{ss}/AP_1 duration ratios. As with the results for AP_2 , cooling decreases the ratio although a higher ratio is always maintained at the base than at the apex.

The influence of ouabain on the AP_{ss}/AP_1 duration ratios

is shown as filled symbols in Fig. 4. The results are more difficult to interpret than those from the cooling experiments. Thus the marked shortening of the action potentials (including AP_1) produced by inhibitory doses of ouabain may give a fairly constant AP_{ss}/AP_1 duration ratio even when both AP_{ss} and AP_1 are substantially shortened. This applies to the apex results. In the case of the base, there is a decrease in the AP_{ss}/AP_1 duration ratio. This means that the AP_{ss}/AP_1 duration ratios become more similar in the presence of ouabain. If this effect is sufficiently large it could lead to a reduction or to inversion of the T wave.

Implications of the results

Several important conclusions can be drawn from our results. (1) Since the first action potentials from the base and apex are very similar in duration it is likely that the ionic currents involved are the same in the two regions. (2) It therefore follows that the differences in duration that occur during normal beating, and that are responsible for determining the polarity of the T wave, develop as a consequence of activity. The onset of this effect must be very rapid since large differences in duration are evident in the second action potential in a train. The second action potential is always longer than the first at the base. It is sometimes longer at the apex but the AP_2/AP_1 duration ratio is always greater at the base than at the apex. (3) In both base and apex regions subsequent action potentials in a train become shorter but the difference in duration between the two areas is always maintained. At normal temperatures the base AP_{ss}/AP_1 duration ratio is near unity. At the apex it is always significantly less than 1. (4) Because the prolongation of AP_2 is reduced or abolished by cooling and by inhibitory doses of ouabain it is possible that the effects are attributable to the response of the Na-K exchange pump to repetitive activity. This response, or its consequences, must be significantly different in the two regions.

Can we offer any explanation for an increase in action potential duration after increased activity of the Na-K pump? At first sight this may seem to be opposite to the expected effect since activation of outward electrogenic pumping would by itself speed repolarisation. This suggests that the effect may arise as a secondary consequence of ionic concentration changes rather than as a direct result of increased pumping. During activity, the cells will gain Na ions and lose K ions. During a single action potential the concentration changes involved will be small unless the movements occur into very restricted spaces. Narrow extracellular spaces do exist in cardiac muscle⁸⁻¹⁰, and rapid changes in extracellular K^+ concentration may occur in them^{11,12}. In particular, $[K^+]$ in the space will increase during activity. It is possible therefore that this increase in $[K^+]$ is responsible for activating the Na-K exchange pump.

It remains, however, to explain how this might prolong the action potential. If $[K^+]$ remains elevated, the action potential will shorten as a consequence of the well known effect of $[K^+]_o$ on plateau duration¹³. To obtain prolongation we would have to suppose that the pump can transiently reduce the $[K^+]$ in the small extracellular spaces or clefts to levels below normal. The action potential would then be prolonged¹⁴.

This interpretation of our results is clearly very tentative. We have no direct evidence that cleft $[K^+]$ is reduced below normal after the first action potential. Moreover, it may be very difficult to test the idea directly as even K^+ -sensitive microelectrodes are not likely to detect the $[K^+]$ level in very narrow cleft spaces. They are more likely to monitor the $[K^+]$ level in the larger extracellular spaces in the preparation.

An alternative view is to postulate that the prolongation of AP_2 is attributable to an increased inward (for example, calcium) current, which is partly (base) or completely

(apex) masked by the shortening effect produced by potassium accumulation. This hypothesis leaves the mechanism of the prolongation unexplained but the difference between the base and apex regions would still be attributable to differences in the capacity of the sodium pump to respond to activity.

Whatever ionic mechanisms form the basis of the effects we have observed, our results do enable us to explain various observations on the T wave. The occurrence of myocardial ischaemia or infarction leads to inversion of the T wave, as does treatment with cardiac glycosides. We can explain these effects as a result of inhibition of the Na-K exchange pump. It is also conceivable that inversion occurs as a consequence of increasing the pump's capacity to respond so that the behaviour of the apex is made to approach that of the base, rather than the opposite. Thus, warming may allow the apex to show prolongation of the action potential during activity (Fig. 3) and so in this manner become similar to the base. This phenomenon could form the basis of T-wave inversion during fever. This possibility also serves to emphasise that it may be misleading to regard T-wave inversions as having a common cause. Any manoeuvre that leads to a decrease in the difference in AP duration will reduce or invert the T wave. This may be

produced either by inhibiting the property of the basal (endocardial) region that allows unexpectedly long action potentials to occur or by stimulating the apex (epicardial) areas to respond to activity in a manner resembling that of the base.

Our results also lead to the expectation that the T-wave should be reduced or inverted after a period of quiescence. This has been observed in whole hearts of frog and tortoise after a short period of quiescence produced by vagal stimulation.

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Intracellular pH and activation of sea urchin eggs after fertilisation

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The intracellular pH of the sea urchin embryo increases 0.3 pH units between 1 and 4 min after fertilisation. The increase in pH is required for initiating development. The increase results from an exchange of extracellular Na⁺ for intracellular H⁺.

ACTIVATION of sea urchin eggs after fertilisation can be separated experimentally into two phases¹. The first, which occurs in the first 60 s after insemination and includes the exocytosis of 1- μ m granules in the cell cortex, seems to be initiated by an increase in intracellular free calcium²⁻⁴. The second, which begins 5 min after insemination and includes increased protein synthesis, commencement of DNA synthesis and other biosynthetic events, seems to result from a nonspecific and pervasive event⁵. The first phase is not a prerequisite for the second, since incubation of eggs in mM concentrations of ammonia^{5,6} or treatments altering the egg surface^{7,8} will start biosynthesis in the absence of the cortical granule exocytosis. This second phase of activation has been shown⁹ to have an obligatory sodium requirement only during the first 5-10 min after insemination at 15 °C. If eggs are transferred to sodium-free seawater between 1 and 5 min after fertilisation, there is no activation of development; however, if sodium (>2.5 mM) is added, development resumes^{9,10}.

Here we present evidence that ammonia and sodium are agents in an efflux of H⁺ ions (the 'fertilisation acid') that occurs after fertilisation. This acid represents H⁺ ions and not a weak organic acid¹¹, and the result of this efflux is

increased intracellular pH. Our data indicate that in normal fertilisation, the hydrogen efflux is mediated through a sodium-hydrogen facilitated transport system in which a transient period of sodium influx is coupled to a period of hydrogen efflux. The latter can also be induced directly by ammonia or other organic amines, in the absence of extracellular sodium. It is this hydrogen efflux, with the resultant change in intracellular pH, and not the sodium influx, that is the prerequisite for metabolic activation.

We suggest that the suppression of metabolism of the unfertilised egg is a result of maintaining a low intracellular pH and that the rapid efflux of hydrogen ions after fertilisation by way of the sodium-coupled exchange raises the intracellular pH. This change allows the increased metabolism referred to as egg activation.

Sodium-proton exchange

We followed acid release by measuring the extracellular pH of a stirred 5% suspension of *Strongylocentrotus purpuratus* eggs (17 °C), using a Corning Model 12 expanded scale pH meter with recorder. After acid efflux was complete, the solution has titrated back to the original pH with standardised sodium hydroxide to determine the amount of acid released. We used two different experimental systems for studying H⁺ release. In the first, normal eggs were suspended in seawater and fertilised by addition of sperm or artificially activated by addition of various amines. The second system, which we refer to as 'choline-arrested eggs', is similar to that described by Chambers for demonstrating the sodium requirement for egg activation^{9,10}.

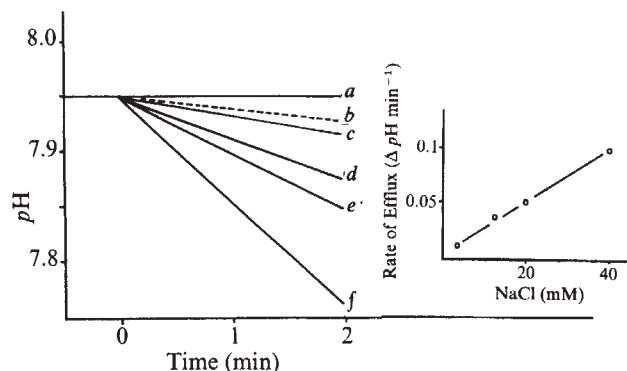


Fig. 1 Acid efflux from eggs as a function of sodium concentration (17 °C). A 2% suspension of eggs was fertilised in seawater, resuspended after 60 s in sodium-free seawater and washed twice. Sodium or lithium chloride was added at zero time to these 'choline-arrested eggs' at the following concentrations and the initial (0–2 min) acid release measured with a Corning expanded scale pH meter and attached chart recorder *a*, 0.1 mM Na; *b*, 100 mM Li; *c*, 3 mM Na; *d*, 10 mM Na; *e*, 20 mM Na; *f*, 40 mM Na. Inset is the slope (rate of efflux against time) plotted against sodium chloride concentration. The relationship between pH and H^+ ion concentration is approximately linear over these small pH ranges.

Eggs were fertilised in normal seawater and then washed and resuspended in sodium-free seawater (choline substituted) 1 min after fertilisation. We then sedimented the eggs rapidly 50 s after insemination in a hand centrifuge, resuspended them in sodium-free seawater at 70 s, and then sedimented and resuspended them again in sodium-free seawater. These eggs have thus undergone a complete cortical exocytosis.

The release of protons by choline-arrested eggs can be linked directly to sodium. Varying amounts of sodium were added to choline-arrested eggs and the rates of H^+ efflux measured. The rate of H^+ efflux is linearly dependent on Na^+ concentration (Fig. 1). Although lithium replaces sodium^{9,10}, it is at least 50 times less effective in mediating the efflux.

To test further the nature of the sodium requirement for hydrogen ion efflux, the effects of the diuretic drug amiloride (3,5-diamino-6-chloropyrazinoyl-guanidine) were determined. This compound inhibits passive sodium transport across toad bladder membranes and has no effect on Na^+-K^+ ATPase pump¹². Eggs were fertilised and after 1 min were transferred to the Na^+ -free choline-substituted seawater. When sodium (40 mM) was added in the absence of amiloride, acid efflux was induced (Fig. 2) and the eggs were activated, as evidenced by subsequent cell division. When 40 mM NaCl was added to choline-arrested eggs in the presence of amiloride (1×10^{-4} M), no acid efflux was observed (Fig. 2); these eggs were not activated and did not divide.

Previous work in our laboratory using eggs activated by ionophore A23187 suggested that about 25% of the H^+ release is independent of sodium¹³. If this part of acid release occurs early (that is, during the first 60 s after insemination), we would not observe this Na^+ -independent acid release with choline arrested eggs. We tested this possibility by fertilising eggs in the presence of 1×10^{-4} M amiloride. There was an initial phase of acid release beginning at 30 s and ending at 60 s, equivalent to 20% of the total acid; 80% of the efflux was prevented (Fig. 3). An early phase of acid efflux (30–60 s after fertilisation) thus accounts for 20% of the total hydrogen release and is independent of Na^+ and insensitive to amiloride. The second phase requires sodium and is sensitive to amiloride.

We had previously found that the various amines which activate metabolism also induce acid release (our unpub-

lished results). The induction of acid release by ammonia (10 mM NH_4Cl , pH 8) is depicted in Fig. 2. The efflux is insensitive to amiloride. Similar results were obtained with procaine (10 mM, pH 8).

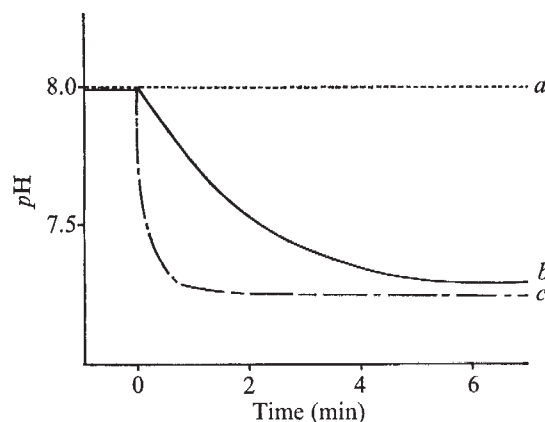
To test whether Na^+ ions are transported across the plasma membrane in exchange for H^+ ions, ^{22}Na influx and H^+ efflux were measured during the first 20 min of development. To measure ^{22}Na uptake, a 1% suspension of eggs was incubated in low-sodium seawater (choline substituted) containing 25 mM sodium and $0.75 \mu Ci ml^{-1}$ of ^{22}Na . Uptake was measured for 15 min, the eggs were then fertilised and changes in sodium influx (see legend to Fig. 4) determined at 1-min intervals. ^{22}Na accumulation was first detectable 1 min after fertilisation, continued for 6 min and then levelled off (Fig. 4). The same time course is seen for acid efflux in choline-substituted seawater containing 25 mM sodium. $^{22}Na^+$ accumulation occurs only during this interval. Its termination does not result from an equilibration of internal and external ^{22}Na ; when isotope was added 10 min after fertilisation in the same conditions there was minimal isotope uptake (100 c.p.m. as opposed to 5,000).

The H^+ efflux in 25 mM Na^+ -seawater was measured in a separate experiment. A 1% suspension (5 ml) was fertilised and after 10 min the eggs were centrifuged and the supernatant was back-titrated to the original pH with standardised NaOH. The Na^+ -dependent portion of the hydrogen efflux (that is, 80% of the total efflux) for 1 ml of a 1% suspension of eggs in seawater pH 8.0 was 4.80×10^{-8} mol and the calculated sodium influx was 4.76×10^{-8} mol. This sodium influx is in agreement with that reported by Chambers¹⁴ and the data indicate a 1:1 exchange of sodium for hydrogen in these conditions.

Amiloride (1×10^{-4} M) rapidly blocked ^{22}Na uptake. When added to eggs 50 s after fertilisation, there was no ^{22}Na accumulation (Fig. 4). The 1:1 stoichiometry and inhibition of both acid efflux and Na^+ influx by amiloride indicates a passive, coupled facilitated transport system with the efflux of each H^+ ion coupled to the influx of a Na^+ ion.

At constant sodium concentration (0.46 M in seawater) the Na^+-H^+ exchange is dependent on external H^+ ion concentration. Confirming earlier results¹¹, we found that the rate and extent of acid efflux was directly related to the extracellular pH. Total efflux was approximately $10 \mu mol$ per ml packed cells when fertilisation was at pH 9 and $6 \mu mol ml^{-1}$ at pH 8. Below an external pH of 6.5 there was no detectable acid efflux and also no activation of development.

Fig. 2 Effect of amiloride on sodium-induced acid efflux from choline-arrested eggs (17 °C). A 5% suspension of choline-arrested eggs was prepared as described in the legend to Fig. 1. The pH of the suspension was then recorded following addition at zero time of 40 mM NaCl—(b), 10 mM NH_4Cl --- (c), or 40 mM NaCl with 1×10^{-4} M amiloride present - - - - (a). Amiloride had no effect on acid release induced by the ammonia addition.



pH of cytoplasm

The observed acid efflux is quite large and should be reflected by an increased intracellular pH. (If the egg cytoplasm were unbuffered, addition of $6 \mu\text{mol}$ of H^+ to 1 ml of cell water at neutral pH would decrease the pH to 2.2.) An estimate of any total cytoplasmic pH changes was made by homogenising eggs before and after insemination and measuring the pH of the homogenate. A 5-ml sample of a 5% suspension of eggs was washed once in unbuffered (bicarbonate free) artificial seawater, resuspended in 5 ml of 0.5 M KCl and then disrupted with 10 strokes in a Dounce homogeniser. The pH was then measured immediately on the expanded scale pH meter with constant stirring. At an external pH of 8.0, the pH of unfertilised egg homogenates (13 separate batches of eggs) was $6.48 (\pm 0.01)$ and in homogenates prepared from embryos 10 min after fertilisation (9 separate batches of eggs) was $6.76 (\pm 0.02)$, a change in pH of 0.28.

Most of this pH shift can be attributed to the efflux of

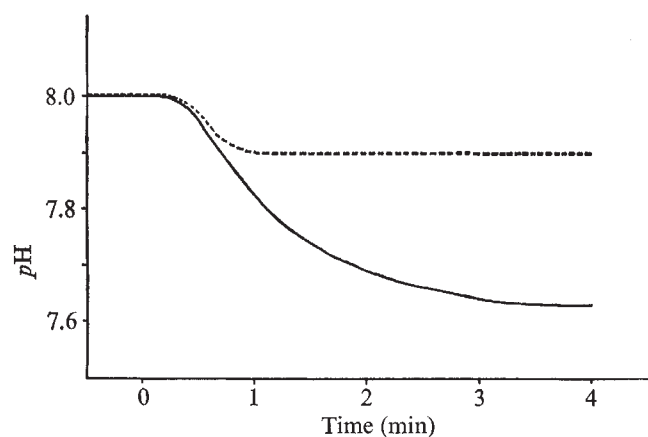


Fig. 3 Acid efflux following fertilisation of eggs in amiloride (17°C). Eggs (2% suspension) were suspended in choline-substituted seawater containing 50 mM sodium chloride with or without $1 \times 10^{-4} \text{ M}$ amiloride. The eggs were then fertilised with sperm at zero time and acid release determined as described (see Fig. 1 legend). The low sodium seawater was used since amiloride is insoluble at higher sodium concentrations. Curves as in Fig. 2.

hydrogen ions already present in the egg, as indicated by titration experiments in which an amount of base equivalent to the released acid ($0.37 \mu\text{mol}$ per 5 ml of a 5% egg suspension) was added back to homogenates of unfertilised eggs (5% egg suspension homogenised in 0.5 M KCl). The pH of this homogenate rose from 6.54 to 6.73 , which is identical to the pH change seen after fertilisation. The bulk of the buffering capacity of egg homogenates is probably in small molecules, and not in heat-labile proteins. The buffering capacity of an egg homogenate that is boiled is in the $10,000g$ supernatant fluid.

The pH of homogenates of fertilised eggs prepared at various times after fertilisation is shown in Fig. 5. There was an initial decrease in pH between 30 and 60 s, possibly related to the Na^+ -insensitive phase of acid release. Then, at 1 min and continuing until 4 min , the pH increased to 6.74 and remained unchanged thereafter. The kinetics correspond extremely well with the time course of H^+ release in seawater.

Implications for development

As already mentioned, it has been shown previously that activation of development requires Na^+ in the extracellular

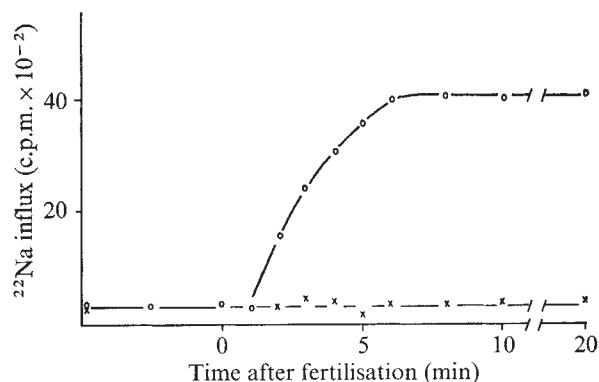
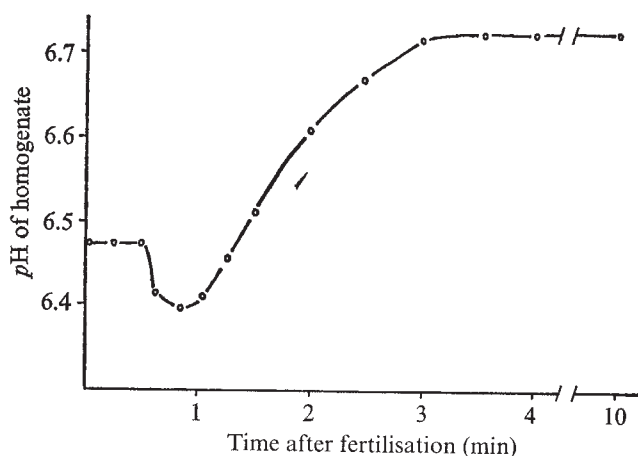


Fig. 4 ^{22}Na influx after fertilisation (17°C). A 1% suspension of eggs was suspended in 20 ml of choline-substituted seawater containing 25 mM sodium chloride. The low sodium seawater was used because of low amiloride solubility and to increase the specific activity of the sodium isotope. ^{22}Na ($15 \mu\text{Ci}$) was added 15 min before fertilisation and 2-ml samples were taken at the times indicated. These samples were added to 8 ml ice-cold seawater, quickly washed once in cold seawater, centrifuged, and 1 ml of NCS tissue solubiliser (Amersham/Searle) added to the pelleted eggs. The eggs were allowed to dissolve in NCS, transferred to a toluene-based scintillation cocktail, and then radioactivity measured in a Beckman LS-230 scintillation counter. Zero time indicates addition of sperm. ^{22}Na influx in the absence of amiloride (O) and in the presence of 10^{-4} M amiloride (x).

environment for a $5\text{--}10 \text{ min}$ interval after fertilisation^{9,10}. Our results indicate that this Na^+ requirement for initiating development can be attributed to its effect in raising the cytoplasmic pH. First, the Na^+ uptake is transitory and is linked stoichiometrically to the H^+ efflux. Second, we find that activation is prevented by the concentrations of amiloride ($1 \times 10^{-4} \text{ M}$) which prevent Na^+ influx and H^+ efflux. This inhibition occurs only during the first 5 min after fertilisation, again corresponding to the period of Na^+ influx and H^+ efflux. Thereafter, amiloride has no effect on development (at least through first cleavage), indicating that its effects are specific to the period of Na^+ influx and not some nonspecific or toxic side effect. Third,

Fig. 5 pH of egg homogenates at various times after fertilisation. A 5% suspension of eggs was lightly treated with trypsin to prevent elevation of the fertilisation membrane and generation of the perivitelline space²⁰. This was necessary to eliminate the contribution of seawater in this space to the pH of the homogenate. Samples (5 ml) of the trypsin-treated eggs were inseminated (17°C) and at the times indicated, the eggs were washed once in bicarbonate-free artificial seawater, centrifuged and suspended in 5 ml of 0.5 M KCl . This fertilised egg suspension was homogenised with ten strokes of a Dounce homogeniser and the pH was determined immediately on a Corning expanded scale pH meter.



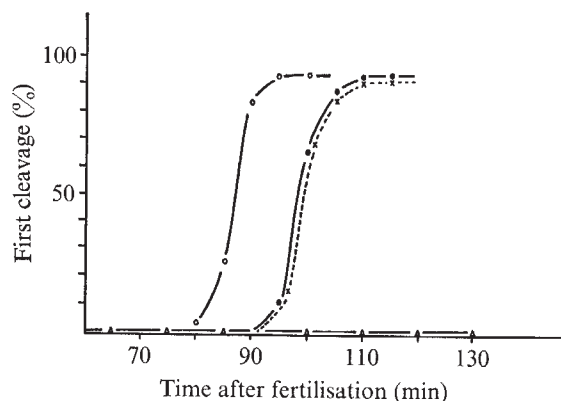


Fig. 6 Capacity of ammonia to substitute for sodium in initiating development. A 1% suspension of eggs was fertilised in seawater and, at 1 min after fertilisation, washed twice in choline-substituted seawater. Aliquots (5 ml) of these choline-arrested eggs were then immediately transferred to 50 ml of seawater (○), sodium-free choline substituted seawater (△), choline-substituted seawater containing 50 mM sodium (●), or choline-substituted seawater containing 5 mM NH_4Cl (×). The embryos were incubated at 17 °C and, beginning at 65 min, 2-ml samples were taken every 5 min and fixed in 5% formalin. The percentage of fully cleaved cells was then determined at each time point.

activation can be prevented by lowering the extracellular pH below 6.5, which prevents acid efflux. As with the amiloride effect, this is seen only during the first few minutes after insemination; embryos develop at least through first cleavage if placed at pH 6.0 after the first 10 min of development.

The fourth and most important observation stems from our finding that ammonia will initiate H^+ efflux in the absence of external sodium (Fig. 2). We found that these concentrations of ammonia can initiate the development of choline-arrested eggs, again in the absence of sodium. The capacity of ammonia to substitute for sodium is shown in the following experiment. Eggs were fertilised and transferred at 1 min to choline-substituted seawater containing either 50 mM sodium or 5 mM ammonia. Control eggs in regular seawater cleave at about 85 min (Fig. 6), whereas eggs transferred to Na^+ -free seawater at 1 min do not cleave (pronuclei do not swell or fuse and no cytasters form). If eggs are transferred to choline seawater with either sodium or ammonia present, however, cleavage occurs—albeit slightly later than the control eggs (the 10-min delay probably results from the presence of choline).

The developmental behaviour of inseminated eggs is identical when either ammonia or Na^+ is present, demonstrating that the important factor in initiating development is not the presence of sodium *per se*. That it is the cytoplasmic pH change that is critical is suggested by the aforementioned induction of H^+ efflux by ammonia and also by the other amines which activate metabolism (1 mM nicotine and 5–10 mM procaine). This H^+ efflux induced by amines is accompanied by a parallel increase in the pH of cell homogenates. These findings, together with the lack of inhibition of amine-induced H^+ efflux by amiloride, indicate that the amines bypass the normal mechanism of coupling H^+ efflux to Na^+ influx. Moreno and Diamond have noted that simple amines can interact with Na^+ channels in frog bladder¹⁸. In eggs, they may be inducing one component only (H^+ efflux) of the vectorial exchange or substituting directly for sodium. Certainly the amines are not increasing cell pH through their action as weak bases. The pH change seen in homogenates prepared from ammonia or procaine-activated eggs can be accounted for completely by the induced efflux of H^+ ions (determined by back-titration of homogenates).

The mechanism of initiating the Na^+-H^+ transport system that begins 60 s after fertilisation is unknown. Alteration of the egg surface by physical, chemical and enzymatic treatments^{7,8} activates metabolism and we have previously implicated a surface glycoprotein in egg activation⁸. This protein is released from the egg surface between 45 s and 120 s after insemination. The close temporal correlation between release of this protein and the beginning of the Na^+ -sensitive H^+ efflux is consistent with the possibility that this protein acts as an inhibitor of the exchange. If so, the observed suppression of metabolism of partially activated eggs by concentrated solutions of this protein⁸ could result from its rebinding to the cell surface and preventing Na^+-H^+ exchange, with a resultant lowering of cell pH. Alternatively, the cell surface proteins may be an additional but separate factor controlling egg activation.

These data should be considered in light of the metabolic transitions that occur at fertilisation. The egg is in a state of metabolic inhibition and is rapidly transformed into a metabolically active cell with striking increases in diverse enzymatic and structural processes. Ca^{2+} is involved in the initial phase³⁻⁴. The second phase, which can be directly activated by amines and which includes increased biosynthesis, seems to result from a nonspecific pervasive change in the cytoplasmic environment. Our data suggest that this pervasive change may be the increase in cytoplasmic pH. Although this change can be initiated independently of the Ca^{2+} phase by the various amines, the amine activation is an incomplete regimen in that a functional mitotic apparatus does not form and cleavage does not occur. All observed changes, Ca^{2+} , pH, and perhaps loss of cell surface protein, seem to be prerequisites for complete development.

Do similar regulatory mechanisms occur in other eggs and other kinds of cells? Calcium regulation of cellular processes is widespread, and the calcium ionophores which activate eggs¹⁷ can also activate various metabolic processes in somatic cells (see, for example, ref. 16). Proton efflux associated with cell activation has so far been described only in sea urchin and *Urechis* eggs¹⁸; in these eggs it is one of the earliest post-fertilisation changes. Extracellular pH can alter cell activity¹⁹, but to our knowledge, intracellular pH has not been seriously considered as an on-off metabolic regulator of other kinds of cells. It is intriguing to speculate that the transition of cells from actively dividing to a resting or G_0 state or from the G_0 to the actively dividing state may be mediated through changes in intracellular pH.

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λ repressor regulates the switch between P_R and P_{rm} promoters

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The DNA region containing the O_R operator, P_R and P_{rm} promoters and their transcription initiations is sequenced. By binding to O_R , repressor turns off P_R , turns on P_{rm} and at higher concentrations turns off P_{rm} , regulating its own synthesis. P_{rm} mRNA is unique in beginning immediately with the initiation of translation, without a preceding leader sequence.

THE right hand operator-promoter region of phage λ is a complex regulatory structure containing the sites of action of at least three proteins which interact with specific DNA sequences: the λ repressor, RNA polymerase and the *tof* or *cro* gene product. The operator itself is a multiple structure containing at least three repressor binding sites^{1,2}. The strongest of these sites overlaps partially with the site to which RNA polymerase binds and from which it initiates rightward transcription of the *tof* gene³. Because of this overlap, repressor binding prevents polymerase binding and therefore blocks transcription.

An additional promoter is located in the vicinity of O_R . This is the P_{rm} promoter, active in the transcription of *cI*, the repressor gene, in the lysogenic state. *In vivo*, the activity of this promoter is dependent on the earlier presence of repressor bound to O_R ⁴. We have previously presented⁵ arguments for the location of P_{rm} within the O_R sequence and predicted that, at high concentrations, repressor should block P_{rm} and therefore turn off its own synthesis, thus acting both as a positive and as a negative autoregulator.

More recently, a mutation in P_{rm} has been localised in the O_R sequence^{5,6} and the autoregulation of repressor synthesis has been demonstrated *in vitro* using as template DNA fragments produced by restriction enzymes.

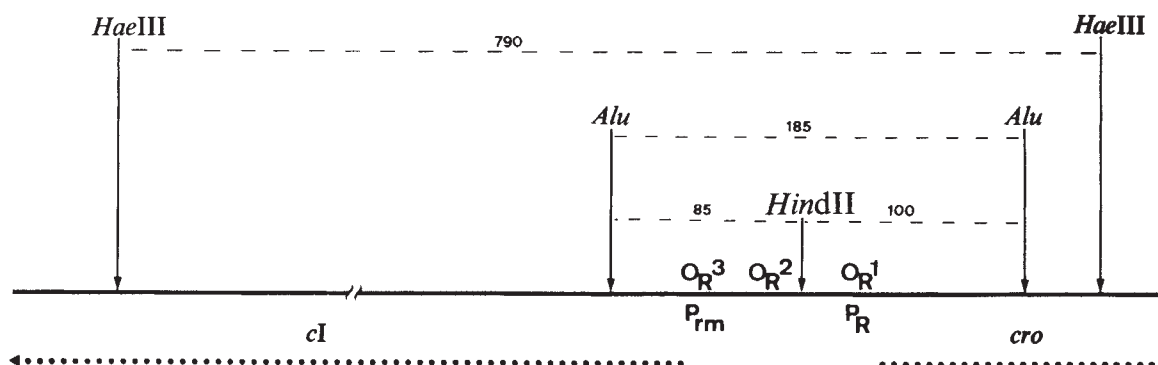
In this article we report the sequence of the DNA region bounded by the *Hind*II cut in O_R and the *Alu*I cut 85 base pairs to the left of it (Fig. 1). This region contains the repressor binding sites O_{R2} and O_{R3} and the beginning of

the *cI* gene. We have used this DNA fragment for direct DNA sequencing using the method of Gilbert *et al.*⁷. In the presence of limited amounts of repressor this fragment or the *Hae*III 790 fragment from the same region are also good templates for P_{rm} transcription. We have sequenced the beginning of the mRNA species stimulated by repressor and we have located it in the DNA sequence. We find that, as predicted by our model, P_{rm} transcription initiates a few nucleotides to the left of O_{R3} . The message begins with AUG and is followed immediately by the codons specifying the amino acid sequence of the amino terminal of the λ repressor protein⁸.

Sequence of the O_R - P_{rm} -*cI* DNA

The restriction endonuclease *Alu*I produces a DNA fragment from the O_R region of λ DNA, which is about 185 base pairs long and contains in the middle the *Hind*II cleavage site which separates the operator primary site O_{R1} from the rest of the operator. The *Alu* 185 fragment can therefore be terminally labelled with polynucleotide kinase, cleaved with *Hind*II and the desired fragment, now labelled only at one 5' end, separated by gel electrophoresis. The shorter fragment, 85 base pairs long, contains the secondary repressor binding sites O_{R2} and O_{R3} and must contain also the beginning of the *cI* gene. We have subjected this fragment, now labelled only at the *Alu* 5' terminal, to the chemical sequencing method of Gilbert *et al.*⁷. Partial methylation with dimethyl sulphate renders the A and G residues sensitive to depurination and chain scission by β elimination. The products are analysed by gel electrophoresis. Strong bands correspond to cleavage at G residues and the weak bands to cleavage at A residues, since the former are methylated six times more rapidly than the latter. Hydrazinolysis in aqueous medium, followed by piperidine-catalysed β elimination, leads to cleavage at C and T residues. An increased specificity of cleavage is obtained by performing the hydrazinolysis in the presence of high ionic strength which leads to preferential reaction at C residues. The sequence is obtained by identifying successive bands present in the purine specific reactions or in

Fig. 1 Restriction endonuclease cleavage sites in the vicinity of O_R . Distances are shown in base pairs. The approximate location of the three repressor binding sites O_{R1} , O_{R2} and O_{R3} and of the two promoters P_R and P_{rm} are indicated. The dotted lines represent transcription of the *cI* gene and of the *cro* or *tof* gene.



the pyrimidine specific reactions. Figure 2 shows that the 3' terminal sequence, though difficult to decipher, corresponds to the previously known sequence of the O_R2 and O_R3 repressor binding sites. The sequence towards the 5' end according to the model should contain the P_{rm} promoter sequence and the initial part of the cI gene.

Repressor stimulated transcription

To identify the initiation of the P_{rm} mRNA in the sequence obtained above, we used the *Hae*III 790 or the *Alu* 185 restriction fragments as templates for transcription. In the absence of repressor we expect P_R transcription to be dominant. In the *Hae* 790 fragment this should yield a

transcription product about 100 bases long. In the *Alu* 185 this product should be about 65 bases long. The P_{rm} transcript from *Hae* 790 should be much longer than the P_R transcript, while if the *Alu* 185 is used as template we expect the P_{rm} transcript to be a maximum of 45 bases long. Furthermore, increasing amounts of repressor in the transcription reaction should block P_R transcription, but stimulate P_{rm} transcription. Figure 3 shows that these expectations are borne out. Using the *Alu* 185 as template, a strong band about 65 bases long is produced in the absence of repressor and disappears at low repressor concentrations. This must be the P_R product. One band, about 35 bases long, is missing in the absence of repressor, appears at low repressor doses and disappears at higher doses. This must be the P_{rm} product. As observed in other systems, glycerol facilitates transcription⁹. We find that at low glycerol concentrations, transcription from P_{rm} is much less efficient even in the presence of suitable amounts of repressor.

Sequence of the P_{rm} mRNA

To verify that the transcription product stimulated by repressor originates from the P_{rm} region and from the correct strand, we eluted the material from the gel and determined its sequence. Figure 4 shows an RNase T_1 fingerprint obtained by electrophoresis on cellulose acetate (pH 3.5), followed by chromatography on a PEI thin layer. Analysis of the spots with pancreatic RNase permitted us to determine their sequence. The 5' triphosphate end, which is barely visible at the lower left corner of the fingerprint, is pppAUG. A rapid inspection of the T_1 oligonucleotide sequences shows that they are in fact complementary to part of the DNA sequence obtained from the *Alu* fragment. The distinctive CACA_nG sequence corresponds to the CT₆GTG in the DNA sequence. The initial pppAUG corresponds to the CAT as shown in Fig. 5. No other ATG occurs in the $\mathcal{L}$ strand in this vicinity. We conclude that the repressor stimulated transcription originates from a unique site, copies the left strand and initiates with AUG.

Initiation of translation

The amino acid sequence near the NH_2 terminal of the λ repressor protein has been determined recently⁸.

The initial 11 amino acids of this sequence fit perfectly with the DNA sequence that we have established and with the sequence of the P_{rm} mRNA. Unlike other known prokaryotic mRNAs, the P_{rm} message initiates immediately with a f-Met codon which is followed by the amino acid sequence of the protein. If the P_{rm} mRNA *in vivo* initiates at the same site as *in vitro*, it contains no leader sequence and thus no provision for a sequence complementary to 16S ribosomal RNA^{10,11}.

Structure of the O_R operator

The sequence upstream of O_R3 shows first of all that there is nothing recognisable as a fourth repressor binding site. The characteristic sequence TATCACC which constitutes the most strongly conserved feature of the repressor recognition sites^{1,2} is missing or is drastically altered. The fourth repressor tetramer which was observed to bind at very high repressor concentrations¹³, was probably due to a relatively nonspecific interaction stabilised by protein interaction with the preceding repressor tetramer. There are then only three functional repressor binding sites in O_R . Recent experiments indicate that this is also the case for O_L (V. P., unpublished). In O_R , the primary site and O_R2 contain sequence elements necessary for the recognition of the P_R promoter by RNA polymerase. Occupation of these sites by the repressor therefore leads to repression of P_R transcription. In consequence these two sites are the targets of mutations which result in constitutive expression of the *tof* gene¹. Smith *et al.*³, using deletions which enter O_R from the left, find that

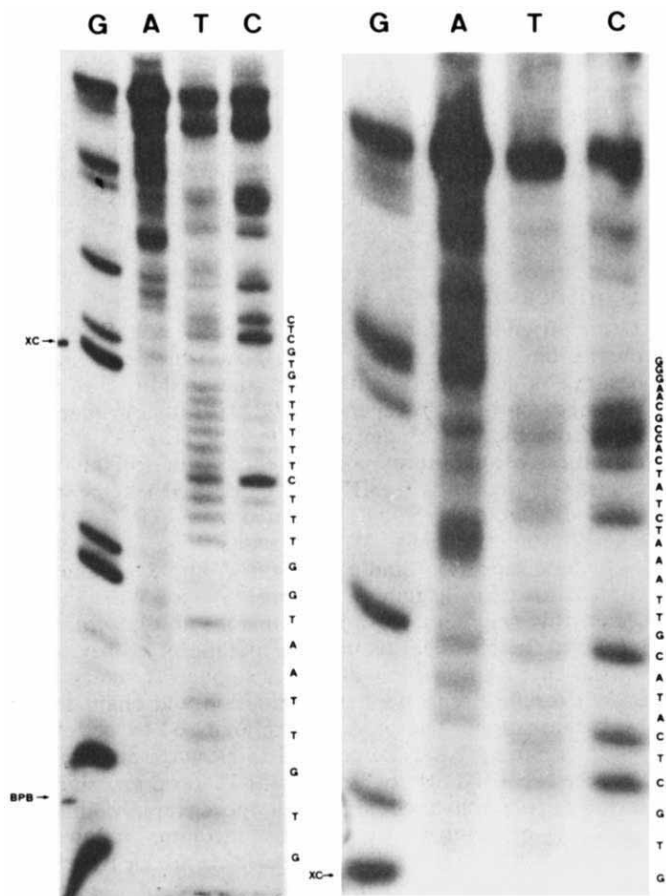


Fig. 2 DNA sequence of the *Alu*-*Hind*II fragment. Purified *Alu* 185 fragments were treated with 2 μ g bacterial alkaline phosphatase for 1 h at 37 °C. After 4 phenol extractions, followed by 4 ether extractions, 1 nmol γ -³²P-ATP (400–800 mCi μ mol⁻¹) and 5–10 units of polynucleotide kinase were added and the reaction mixture was incubated at 37 °C for 1 h. The labelled fragments were cleaved with *Hind*II and the products, now labelled at only one 5' end, were purified by gel electrophoresis on 0.7% Agarose-3% acrylamide gels in 0.09 Tris-borate (pH 8.2). The desired band was extracted from the gel and treated with dimethylsulphate (purine specific cleavage) or with hydrazine either low salt (T specific cleavage) or in 1 M KCl (C specific cleavage), according to the method of Gilbert *et al.*⁷ and of Gilbert and Maxam (personal communication). Preferential cleavage at A is obtained by depurinating the methylated DNA at acid rather than alkaline pH. The products were analysed on a 40 cm gel containing 20% acrylamide, 7 M urea and 0.05 M Tris-borate (pH 8.2). Electrophoresis proceeded at 600 V for about 14 h. One glass plate was removed, the gel was wrapped in plastic film and autoradiographed at -20 °C (left). To obtain better resolution in the upper part of the gel, the glass plate could be replaced and electrophoresis could be continued with little loss in the definition of the bands (to the right, shown at slightly greater magnification). BPB and XC indicate the positions of the marker dyes which comigrate with fragments 10 and 29 bases long, respectively.

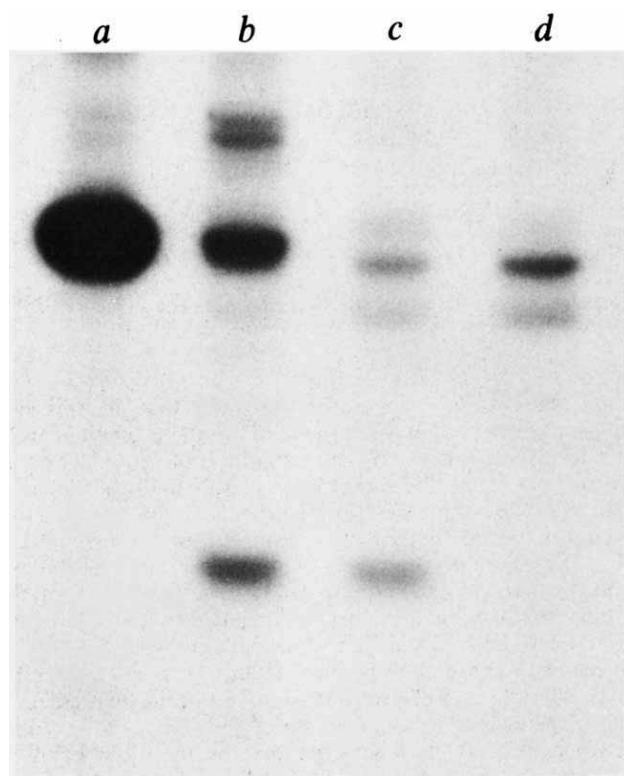


Fig. 3 Turn-on and turn-off of P_{rm} transcription. 1 pmol of *Alu* 185 fragment was incubated at 20 °C for 10 min with (a) no repressor, (b) 0.5 μ l, (c) 2 μ l and (d) 10 μ l of λ repressor in 50 μ l containing 20 mM Tris (pH 7.9), 0.1 mM EDTA, 0.1 mM dithiothreitol, 8 mM $MgCl_2$, 40 mM KCl and 15% glycerol. RNA polymerase (2 μ g or 1.7 U) and ATP (200 μ M final concentration) were added and incubation was continued for 10 min at 37 °C. 1 μ l of 2 mg ml⁻¹ heparin was then added, followed 2 min later by GTP and UTP at a final concentration 10 μ M and α -³²P CTP (100 mCi μ mol⁻¹) at 2 μ M. The reaction was stopped after 10 min at 37 °C by a phenol extraction and ethanol precipitation. The material was analysed on a 12% acrylamide gel containing 7 M urea and 90 mM Tris-borate (pH 8.2). The strong band in (a) is the P_R transcript while the band that appears in (b) and (c) is presumed to be the P_{rm} transcript. The weak band, of roughly the same size as the P_R transcript, which seems to be stimulated by repressor, was also sequenced and found to be due to contaminating traces of a different *Alu* fragment.

the first two sites, but not O_R3 , are required for effective repression.

P_{rm} promoter

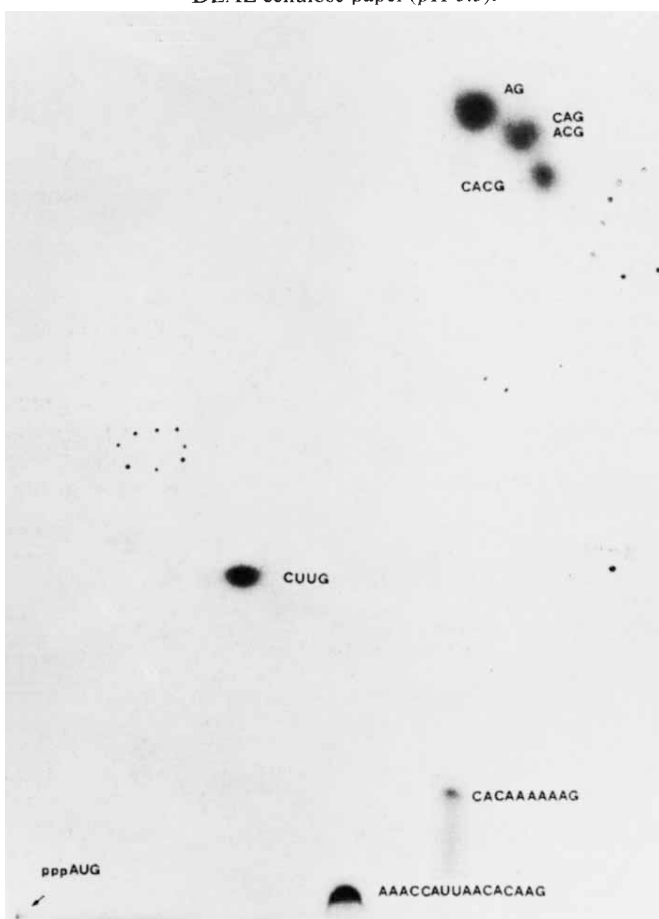
In an earlier article³ we have pointed out a remarkable degree of symmetry centred around the middle of O_R2 . This symmetry is absent in O_L because of the different disposition of the repressor binding sites. As a consequence of this symmetry, O_R contains another sequence similar to that of P_R but with orientation opposite to it. This, we proposed, was the P_{rm} promoter. This conclusion was supported by the finding that a mutation in P_{rm} is located between O_R2 and O_R3 (refs 5, 6).

The symmetry results in substantial sequence homology between the P_R and the P_{rm} region, but it is not perfect. If the P_R and P_{rm} sequences are aligned as in Fig. 6, good agreement is obtained only if half of the sequence is displaced by one base. Therefore, assuming that the P_R sequence is representative of a good promoter, some adjustment needs to be made in order for P_{rm} to resemble it. This probably accounts for the finding that in the absence of repressor, P_{rm} is inactive. By binding to O_R1 , the repressor in some way facilitates the recognition of P_{rm} by the RNA polymerase. We do not yet know the mechanism of this facilitation.

A possible interpretation is suggested by the observation that RNA polymerase is unable to bind to the *Hind*III fragment containing O_R1 (ref. 14) even though the site at which it forms the initiation complex is entirely to the right of the *Hind*III cleavage site (Fig. 7). We conclude that the polymerase must interact with the region of O_R2 in order to bind to the P_R promoter. The symmetric arrangement of P_R and P_{rm} suggests that the same region is required for binding to P_{rm} . This region would then be a non-directional recognition site from which RNA polymerase could move in either direction to form the initiation complex at P_R or P_{rm} . Repressor might activate P_{rm} simply by blocking the stronger promoter P_R and removing a competitive binding site for polymerase. Alternatively the repressor might change the conformation of the DNA in the vicinity of O_R2 - O_R3 , making it possible for the polymerase to recognise P_{rm} .

We do not know yet what particular sequence features at what positions are required for RNA polymerase binding. The substantial variety in the promoter sequences determined so far, suggests that the polymerase can accommodate a wide range of sequences. The fact that single base changes can inactivate promoter sites indicates, however, that in a given sequence, certain nucleotides play a key role. For example, the *sex* 1 mutation in P_L affects a

Fig. 4 RNase T₁ fingerprint of P_{rm} transcription initiation. In this case the template used was the *Hae* 790 fragment. In the transcription reaction the ATP concentration was 200 μ M. The other 3 nucleotide triphosphates were labelled and at 1.7 μ M. A partial transcription product about 45 bases long was eluted from the gel as described previously², digested with RNase T₁ and fractionated in the first dimension by electrophoresis on cellulose acetate (pH 3.5). This was followed by chromatography in the second dimension on a PEI cellulose thin layer with 2 M pyridine formate (pH 3.5) and 7 M urea. The spots were analysed by digestion with pancreatic RNase and electrophoresis on DEAE cellulose paper (pH 3.5).



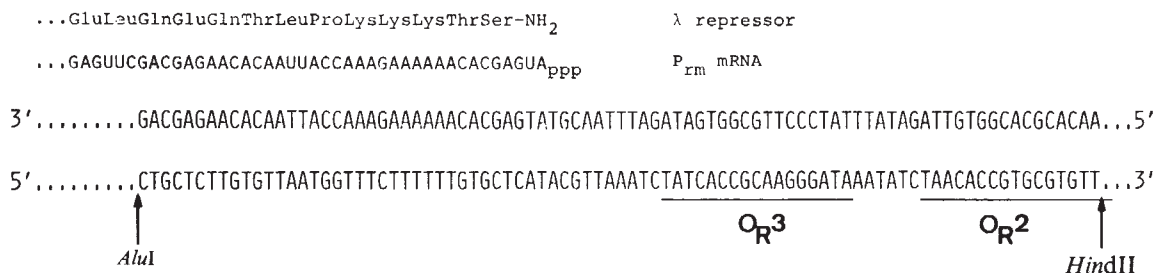


Fig. 5 Sequence of the *Alu*–*HindII* fragment. The arrows indicate the cleavage sites of the two restriction enzymes. The sequence of the P_{rm} mRNA and the corresponding amino acid sequence of the λ repressor are also shown⁸.

GC pair in the position shown in Fig. 6¹. The corresponding position in P_{rm} is also of crucial importance. This is the site where Meyer *et al.*⁶ place the P_{rm} 116 mutation and it is also the site where the P_{rm} sequence goes out of register with that of P_R. Significantly, the effects of the *sex 1* mutation can be compensated by the presence of glycerol in an *in vitro* transcription reaction⁹. This suggests that both in the *sex 1* mutation and in the normal P_{rm}, the limiting step is the opening of the strands by the polymerase to form the so-called “open” complex (for review see refs 15 and 16).

Another possible recognition element is the heptanucleotide which can be discerned, with certain variations, in all RNA polymerase binding sites, 6–7 nucleotides upstream of the transcription initiation site¹⁷. This heptanucleotide, whose canonical form is TATpupuTpu, is

same site *in vivo*, we must conclude that in this case, ribosome interaction with the initiation site on the mRNA is entirely dependent on the recognition of the AUG codon. We do not know the significance of this finding. We speculate, however, that the lack of other stabilising interactions would make the binding of ribosomes less efficient and limit the utilisation of the mRNA. Moreover, attack at the 5' end by *exoV* would immediately inactivate P_{rm} mRNA which would then be expected to have an unusually short functional half life. It is remarkable that the phage has developed such elaborate mechanisms both at the level of transcription and of translation to limit the expression of the *cI* gene in the lysogenic state.

Upon infection of a sensitive cell, on the other hand, the *cI* gene is transcribed from a different promoter, the so-

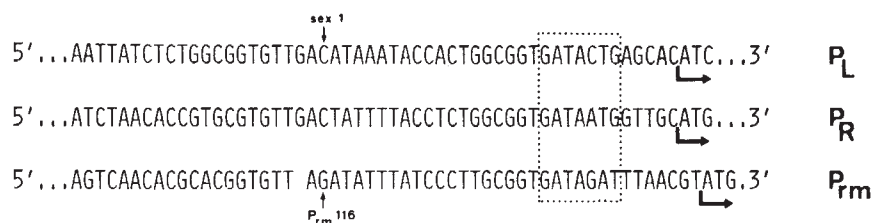


Fig. 6 Comparison of promoter sequences. The λ P_L, P_R and P_{rm} sequences are aligned to emphasise their homology. The arrows indicate the initiation of transcription. Maximum homology is obtained if part of the P_{rm} sequence is displaced by one base. The sites of the *sex 1* and of the P_{rm} 116 promoter mutations⁶ are indicated. The dotted line encloses the heptanucleotide generally found in RNA polymerase binding sites¹⁷.

present in P_R as GATAATG. In P_{rm} it is further changed to GATAGAT.

P_{rm} mRNA has no leader sequence

All prokaryotic mRNAs so far studied contain a stretch of variable length preceding the initiator AUG codon. This stretch has been called the leader and is thought to be important in directing the binding of ribosomes to the translation initiation site¹⁰. In contrast, the P_{rm} mRNA begins immediately with the initiator codon and lacks the polypurine tract which is thought to interact with the 3' end of the 16S ribosomal RNA. If the P_{rm} mRNA begins at the

called P_{re} promoter, located to the right of the *tof* gene⁴. Transcription originating from this promoter has potentially a leader region several hundred nucleotides long which contains the anti-sense sequence of the *tof* gene and includes the O_R sequence. This region, as far as we know, is not translated. In this case, the sequence preceding the initiator codon AUG contains all the features of a typical ribosomal binding site. There is in fact a GGTGAT in the operator sequence which is complementary to the AUCACC close to the 3' end of the ribosomal 16S RNA. The sequence contains a UUU which is also found in several other ribosomal binding sites^{11,18} and, finally, three terminator codons in

Fig. 7 The P_{rm}–O_R–P_R region. The two promoters are shown symmetrically disposed and overlapping the two ends of the operator. In P_R, RNA polymerase forms the tight binding and initiation complex at the site indicated, partially overlapping O_R1. A presumed corresponding site for P_{rm} is shown by a dashed line. The initiation of transcription is indicated by arrows. The entire structure is symmetrically arranged around the centre of O_R2. This is the region where the RNA polymerase might form the initial recognition complex. Repressor bound to O_R1 would then steer the polymerase to the P_{rm} binding and initiation site.



phase precede the initiator AUG codon.

To conclude, we note that *tof* mRNA, like the P_{rm} mRNA, also begins with an AUG. This similarity may not be entirely fortuitous. It is in keeping with the above-mentioned symmetry of the entire O_R region and with the fact that *tof* product, like the *cI* product, acts as a repressor, albeit a weak one. Its sites of action, like those of the *cI* repressor, are O_L where it performs the eponymous function of turning off the *N* operon, and O_R , where it turns off transcription of P_{rm} and of P_R and hence its own transcription¹⁹. In short, the *tof* product may formally be considered a mini version of the *cI* repressor. In the *tof* mRNA, however, the initial AUG is not the start of translation because it is followed two triplets later by a UAA codon in phase.

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letters to nature

The second highly polarised radio outburst in Cygnus X-3

WE detected a second outburst with a high degree of linear polarisation from Cyg X-3 in August 1975, following the alert by Kawajiri¹. The first such outburst was observed in May 1974 (refs 2 and 3). Here we report observations of both polarised outbursts as well as of an unpolarised outburst in January 1975. At 8 GHz the observed polarisation position angle was the same for both polarised outbursts. The Faraday rotation measure associated with the source is much larger than previously reported². We suggest that the orientation of the magnetic field in the source and the rotation measure are both stable with time. In addition, we find that the highly polarised outbursts decay significantly more slowly than the unpolarised outbursts.

All measurements were made with the University of Michigan 85-foot paraboloid operating at frequencies of 8 and 14.5 GHz. The equipment and on-off observing techniques used at 8 GHz have been described previously^{4–6}. Similar equipment and techniques were used at 14.5 GHz. All the observations were made with linear polarimeters except for those made at 8 GHz on May 26 and June 2, 1974 and on August 27–28, 1975, which were obtained with a single-horn circular polarimeter. Observations of Cyg X-3 were alternated with observations of the standard sources Cyg A or DR21 to determine the flux density calibration. Daily averages of the data for the polarised outbursts are presented in Table 1. The total flux density measurements have been corrected for confusion effects by the addition of 0.071 Jy at 14.5 GHz and the subtraction of either 0.73 or 0.49 Jy at 8 GHz for observations made with the linear or circular polarimeters respectively. The standard errors include the statistical noise and the uncertainties in the antenna gain and telescope pointing.

The total and polarised flux densities for August and September 1975 are displayed in Fig. 1. Only the decay phase of the outburst was observed. Observations⁷ at 5 GHz indicate that the maximum flux density occurred on August 22 or 23, 2 or 3 d before our measurements began. From August 26 to September 3, the decay of the total flux den-

sity at 8 GHz was exponential with time. The line through the 8-GHz data points is a least squares fit to the data from August 26–27 to September 1–2 and represents an e-folding time of 86 ± 1 h. The 14.5-GHz data are consistent with the same exponential decay rate. The spectral index from 14.5 to 8 GHz during the exponential decay remained at $\alpha = -0.65 \pm 0.06$ where $\alpha = (\Delta \log S_\nu / \Delta \log \nu)$. The same spectral index appears to fit the data at 5 GHz (ref. 7) which indicates that the source was transparent above 5 GHz from August 24 to September 5. After September 3 the rapid exponential decay ceased and the total flux density slowly and irregularly declined until September 20. By September 10 the flux density seems to have been nearly the same from 5 GHz to 14.5 GHz, corresponding to a spectral index $\alpha \sim 0$.

Table 1 Flux density and linear polarisation variations with time

Date	(UT)	S_ν (Jy)	P (%)	Position angle (degrees)
8.0 GHz				
1974				
May	26.5	4.18 ± 0.35	9.7 ± 0.8	80 ± 2
June	2.4	1.70 ± 0.35	7.0 ± 1.8	87 ± 7
1975				
August	25.3	7.34 ± 0.12	5.8 ± 0.5	75 ± 2
	26.3	6.32 ± 0.14	7.6 ± 0.7	80 ± 2
	27.1	5.48 ± 0.08	8.4 ± 0.5	80 ± 2
	28.1	4.25 ± 0.18	8.4 ± 0.5	86 ± 2
	29.0	3.21 ± 0.09	11.6 ± 1.6	83 ± 4
September	2.1	1.04 ± 0.03	12.5 ± 2.7	87 ± 6
	3.0	0.90 ± 0.06	17.1 ± 5.1	97 ± 7
	5.2	0.46 ± 0.04	8.6 ± 9.5	
	8.1	0.54 ± 0.04	19.8 ± 7.0	83 ± 7
	10.1	0.36 ± 0.08	23.4 ± 14.8	84 ± 16
	11.0	0.47 ± 0.06	18.4 ± 14.6	88 ± 14
	17.1	0.27 ± 0.05	25.6 ± 18.5	143 ± 18
14.5 GHz				
1975				
August	27.2	3.69 ± 0.25	12.6 ± 2.1	146 ± 5
	28.2	2.65 ± 0.13	17.4 ± 2.5	154 ± 4
	29.3	2.04 ± 0.16	17.7 ± 4.9	145 ± 7
September	3.1	0.64 ± 0.07	27.9 ± 14.9	151 ± 19
	9.1	0.58 ± 0.10	22.2 ± 15.3	2 ± 23
	12.0	0.33 ± 0.11	15.3 ± 28.8	

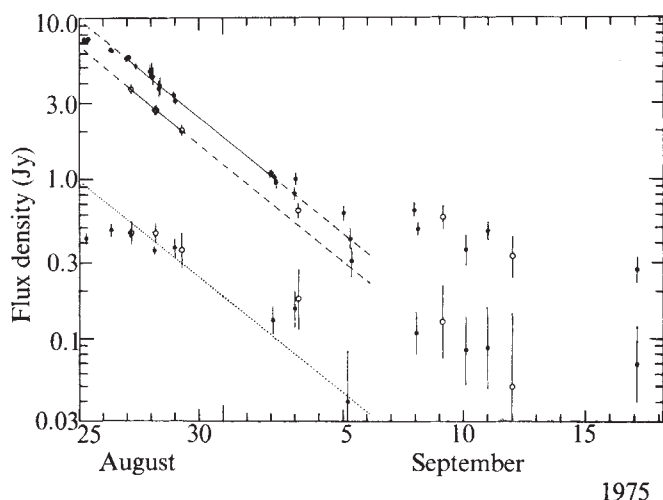


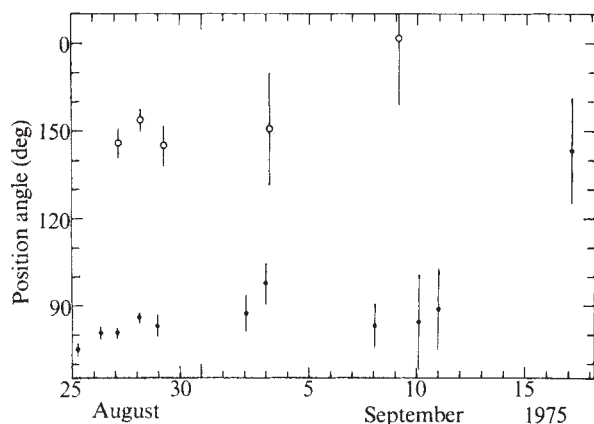
Fig. 1 Semilog plot of total and polarised flux density against universal time at 8.0 (●) and 14.5 (○) GHz for Cyg X-3. The total flux density measurements in the upper portion of the plot are 40-min averages of the data. The polarised flux density measurements in the lower portion are daily averages. The two lines through the total flux density measurements are fits to an exponential decay with time for the data where the lines are solid. The dotted line through the polarised flux density measurements represents a constant 10% degree of linear polarisation for the exponential decay of the total flux density at 8 GHz.

It has been noted that the decay of the first September 1972 outburst evolved from an exponential to a power-law time dependence with the exponential phase lasting longer at higher frequencies⁸. The same phenomenon may have occurred during this outburst. The irregular flux variations and the apparent change in the spectral index indicate, however, that renewed injection or acceleration of particles may have obscured the decay of the outburst after September 3. Hence, fits to the data extending after September 3, such as the one made at 5 GHz (ref. 7) may not give a true indication of the decay process.

No circular polarisation was detected in either polarised outburst. A 2σ upper limit of 0.5% for the degree of circular polarisation was obtained on August 27–28, 1975. Our measured degree of circular polarisation of $0.4 \pm 0.4\%$ for the May 1974 outburst agrees with the upper limit of 0.5% obtained by Seaquist *et al.*²

During the August 1975 outburst the degree of linear polarisation increased to $> 10\%$ at both frequencies, as illustrated in Fig. 1. The level of polarisation at 8 GHz is comparable to that observed by us and by Seaquist *et al.*²

Fig. 2 Linear polarisation position angle against universal time at 8.0 (●) and 14.5 (○) GHz. The points represent daily averages of the data.



during the May 1974 outburst. Although the total flux density was already decreasing when we started observing on August 25, 1975, the polarised flux density did not begin to decrease until August 27. The increase in the degree of polarisation with time, and the fact that the degree of polarisation was significantly higher at the higher frequency (14.5 GHz) are both similar to the pattern observed during the May 1974 outburst^{2,3}.

The position angles of the linear polarisation for the August 1975 outburst are shown in Fig. 2. The average position angle observed at 8 GHz from August 25 to September 3 of $81.1 \pm 1.0^\circ$ is the same within uncertainties as the value of $80.5 \pm 2.2^\circ$ observed by us for the May 1974 outburst. The position angle may have changed at 8 GHz by $\sim 10^\circ$ during the 1975 outburst. The average position angle observed at 14.5 GHz was $150 \pm 3^\circ$. If the difference in position angle is caused by Faraday rotation, the rotation measure must be at least $-1,265 \pm 56 \text{ rad m}^{-2}$. This value is more than twelve times that derived from data at the frequencies 2.7 and 8.1 GHz (ref. 2). Observations at 4.2 GHz for the May 1974 outburst³ also indicate that the original determination of the rotation measure was too small because of the m -ambiguity in the amount of rotation at 2.7 GHz. The excellent agreement of the observed position angles for the two polarised outbursts strongly suggests that the rotation measure and intrinsic position angle were the same for both outbursts. If the position angles of the two outbursts were completely independent, the probability that the difference would be not more than 0.6° is 0.7%. The probability that the difference would be not more than the quadratically summed error of 2.4° is 2.7%. We have fitted the observed position angles from both outbursts together and find that a rotation measure of $-1,233 \pm 10 \text{ rad m}^{-2}$ is consistent with all the data. The intrinsic position angle of the linear polarisation was $0 \pm 3^\circ$.

The stability of the 8-GHz polarisation position angle during the outbursts and between the first outburst and the second indicates that most of the Faraday rotation must occur in a region which is unaffected by the evolution of the outbursts. It is extremely unlikely that the rotation measure within the expanding emitting region would be the same for two separate bursts or that the internal rotation measure would not change as the emitting region expanded. The region producing the observed Faraday rotation could be either the long path through the Galaxy between Cyg X-3 and the Earth or a volume around Cyg X-3 in which material has accumulated from previous activity in the source. The sign of the rotation measure is the same as that observed for other sources in the Cygnus direction through the Galaxy, although the magnitude is much larger^{9,10}. If the distance to Cyg X-3 is taken as 11 kpc (refs 11 and 12), the observed rotation measure could be produced by an average longitudinal component of the magnetic field of 10^{-8} gauss and an average electron density of 0.14 cm^{-3} . These are reasonable values, especially since the source is located on the far side of the Cyg X complex of H II regions.

The similar degrees of polarisation and the agreement between the polarisation position angles for the two outbursts suggest that the structure of the magnetic field in Cyg X-3 was the same for both outbursts. The periodicity observed in the radio^{8,9}, infrared¹³ and X-ray¹⁴ emissions indicates that Cyg X-3 is a rotating or revolving system with a period of 4.8 h. The absence of large changes in the polarisation position angle data indicates that the magnetic field in the emitting region is oriented near a direction parallel or perpendicular to the angular momentum axis of the object.

Since the intrinsic position angle of the linear polarisation produced in an optically thin source is perpendicular to the transverse projection of the magnetic field, the axis of

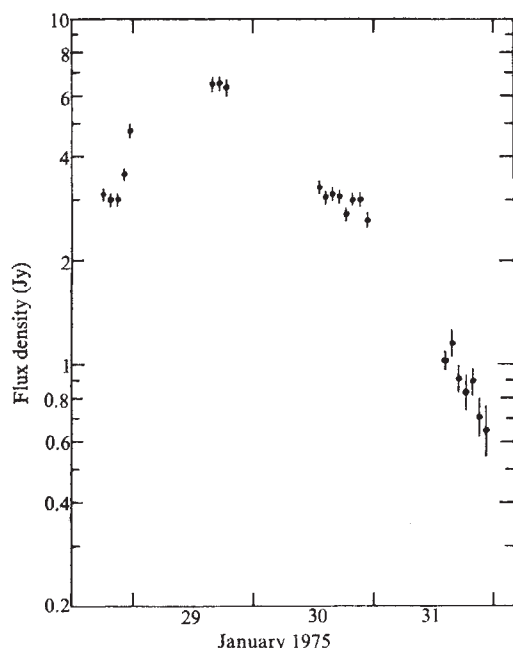


Fig. 3 Semilog plot of total flux density against UT at 8.0 GHz for Cyg X-3. The points represent 40-min averages of the data.

rotation or revolution of the system should be near a position angle of 0° or 90° . Any model for the radio emission from this source, especially those involving expansion where the particles control the magnetic field^{15,16}, must explain the observed stability of the field structure.

Observations at 8 GHz of an unpolarised outburst in Cyg X-3 during January 1975 are shown in Fig. 3. The average degree of linear polarisation from our 23 measurements was $1.3 \pm 0.5\%$ at a position angle of $66 \pm 12^\circ$. The characteristic time for the decay of the total flux density was ~ 27 h which is similar to the decay times found for the other unpolarised outbursts^{5,17}. It is noteworthy that the two highly linearly polarised outbursts have decayed significantly more slowly than all the other observed outbursts, of which four are known to have been unpolarised^{2,6,18,19}.

If there is a correlation between the decay rate and the existence of linear polarisation, it is an important feature which must be explained by any model for the radio outbursts in Cyg X-3. For instance, there is a natural correlation between the decay rate and the amount of linear polarisation if the absence of polarisation in the unpolarised events is caused by Faraday depolarisation and the energy losses of the radiating particles arise from thermal bremsstrahlung. This energy loss mechanism has been suggested to explain the exponential nature of the decay⁵. The difference in the amount of thermal matter required for polarised and unpolarised outbursts is much larger, however, than the difference required to explain the different decay rates. On the other hand, it is not obvious why a correlation between the decay rate and the state of polarisation would exist for expanding source models in which the energy losses are due to adiabatic expansion¹⁵ or to both adiabatic expansion and synchrotron radiation¹⁶. Source models for Cyg X-3 must account for the smaller degree of polarisation at lower frequencies during the polarised outbursts. For example, the ratio of the degree of polarisation at 8 GHz to that at 14.5 GHz was 0.54 ± 0.06 . It has been suggested that this depolarisation effect arises from Faraday depolarisation². This explanation is, however, hard to reconcile with the stability of the polarisation position angle observed at 8 GHz.

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Competition of neutrino and gravitational radiation in neutron star formation

WE look here into the possibility that neutrino radiation (rather than gravitational radiation) may be the dominant way by which non-radial pulsations are damped out in a collapsing star. If true, such a result implies that estimates and hopes to detect gravity waves from supernovae explosions may have been overly optimistic.

It has been known for some time that neutron stars and black holes are probably the collapsed central remnants of a supernova explosion. These objects presumably originate from the collapse of the cores of sufficiently massive ($M \gtrsim 7M_\odot$) stars¹, following the cessation of thermonuclear burning. Although at the present no completely consistent detailed theory exists showing exactly how the collapse of the core and the subsequent supernova explosion take place, there does exist a general model² for the final stages of stellar evolution and supernovae explosions. According to this, the electrons³ of a sufficiently massive stellar core, due to the high density and temperature, get absorbed by the protons, through $p + e^- \rightarrow n + \nu$. At the same time, huge numbers of neutrinos, resulting from this and other thermal processes (pair annihilation, plasma decay, bremsstrahlung) (refs 3 and 4) are emitted, taking away most of the gravitational energy of the collapse. These neutrinos possibly drive the ejection of the overlying stellar mantle, while the neutron-rich core further collapses to a condensed remnant.

The leftover remnant is of very high density (10^{14} – 10^{15} g cm⁻³) and its radius will eventually be only a few times its Schwarzschild radius. This implies that general relativistic effects may be important in situations involving neutron stars. In particular, the possibility of gravitational radiation emission from non-radially pulsating neutron stars has been extensively examined and it has been shown that gravitational waves can carry away the pulsational energy, thus damping out the star's oscillations. (Such non-radial pulsations presumably might occur during the collapse and formation of the neutron star.) Some workers have estimated⁵ that the energy emitted in gravitational radiation in such an event would be sufficient to trigger existing or future gravitational radiation detectors. Consequently one might expect detectable gravitational wave pulses to be associated with supernovae explosions from the non-radial pulsations of the newly formed neutron stars.

As previously discussed, however, the dominant mechanism by which energy is carried away from the collapsing core is by neutrinos rather than gravitational radiation, the latter coming into play only at the very late stages of the collapse. This implies that neutrino radiation might also contribute to the decay of the non-radial oscillations of the collapsing core and the newly formed neutron star, possibly damping out these oscillations much faster than gravitational radiation.

To give a more quantitative answer to this question, the effects of neutrino radiation on the non-radial oscillations of such objects have been examined (D. Kazanas and D. N. Schramm, unpublished). The oscillations are assumed small enough so that the linear approximation in the perturbation is sufficient. The equilibrium configuration is assumed to be static (the velocity of the fluid $\bar{u} = 0$ when in equilibrium) and homogeneous ($\nabla p_0 = 0$). (The subscript zero denotes unperturbed quantities.) Its mass was taken to be $0.75M_\odot$ (to avoid an overestimate of the central temperatures) and it was assumed to obey an ideal gas law (not unreasonable for such a hot neutron gas). Together with the density, these completely specify the equilibrium configuration (the radius, and temperature and

for $\rho = 10^{15} \text{ g cm}^{-3}$. For lower densities gravitational radiation is essentially absent and thus $\tau_G \gg \tau_\nu$.

The implication of the above result is easy to see. It seems that neutrino radiation, by more rapid damping of the non-radial oscillations of a newly formed neutron star in a supernova explosion, would hinder gravitational radiation, thus reducing the possibility of its detection.

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Table 1 Variation of central temperature and radius with density for a neutron star

$\rho(\text{g cm}^{-3})$	$T(10^9 \text{ K})$	$R(\text{km})$
10^{12}	84	70.7
10^{13}	181	32.8
10^{14}	389	15.2
10^{15}	839	7.0

pressure distributions). Table 1 gives the radii and central temperatures for such a configuration for four different density regimes. (Of course, the figures for the highest density are only formal, because the equation of state then differs significantly from that of an ideal gas.) Assuming also an $\exp(i\sigma t)$ time dependence for all perturbed quantities, one can estimate a damping time scale from the neutrino radiation. This will be, approximately, the total kinetic energy of the perturbation, E_{tot} , divided by the rate at which energy is lost by neutrino radiation, dE_ν/dt . This neutrino damping time scale is, as shown elsewhere (D. Kazanas and D. N. Schramm, unpublished), given by

$$\frac{1}{\tau} = \frac{dE_\nu/dt}{E_{\text{tot}}} = \frac{1}{2} \frac{(\Gamma-1) \int \delta L (\delta \rho / \rho_0)_a dV}{(\sigma_a^2/2) \int (\delta r)_a^2 \rho_0 dV}$$

where the integrals extend over the volume of the configuration and where Γ is the adiabatic index, δL the perturbation of the neutrino luminosity in $\text{erg s}^{-1} \text{ cm}^{-3}$, and the subscript a denotes that the quantities have to be evaluated for the corresponding adiabatic solution (with $L = 0$). The expression for the quantities $(\delta r)_a$ and $(\delta \rho / \rho_0)_a$ and values for σ_a can be found in the literature⁶.

One can now evaluate the damping times with δL corresponding to a particular neutrino production mechanism. Using only the dominant thermal neutrino production process, which for the densities and temperatures involved, appears to be pair annihilation, one can obtain upper limit estimates of the damping times due to neutrino radiation. The results for the four different density regimes are shown in Table 2.

It is interesting to compare these to the corresponding damping times due to gravitational radiation. A variational calculation⁷ gives $t = 10 \text{ s}$ for $\rho = 10^{14} \text{ g cm}^{-3}$ and $t = 0.4 \text{ s}$

Table 2 Density dependence of neutrino-damping time

$\rho(\text{g cm}^{-3})$	$\sigma_a(\text{s}^{-1})$	$\tau(\text{s})$
10^{12}	1,531	0.7
10^{13}	4,842	5×10^{-2}
10^{14}	15,310	10^{-3}
10^{15}	48,416	2×10^{-6}

Detection of interstellar HN^{13}C

INTERSTELLAR hydrogen isocyanide (HNC) was first tentatively identified¹ on the basis of a calculated frequency for the $J=1 \rightarrow 0$ transition of the most abundant isotopic species $\text{H}^{14}\text{N}^{12}\text{C}$. The observed interstellar line was quite strong and widespread in the galaxy. A more definitive identification of the observed U90.7 emission line (90,663.6 MHz)² by searching for other isotopes was not possible because of the large uncertainties in the calculated molecular structure of HNC, and the corresponding large uncertainties in the calculated transition frequencies for $\text{H}^{14}\text{N}^{13}\text{C}$ and $\text{H}^{15}\text{N}^{12}\text{C}$. Hydrogen isocyanide is very short lived in terrestrial laboratory conditions, and therefore it has proven difficult to measure the rotational spectrum directly. The $J=0 \rightarrow 1$ transition has, however, been recently measured independently by three groups³⁻⁵ and the frequency of the major isotope is in excellent agreement with that of U90.7. The laboratory spectroscopy has also

Fig. 1 HN^{12}C (a) and HN^{13}C ($\times 10$) (b) in W51. Note the HN^{12}C line is broader: resolution = 1 MHz ($\approx 3.3 \text{ km s}^{-1}$).

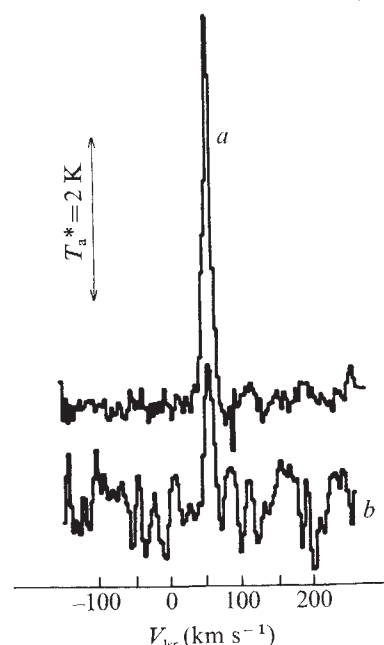


Fig. 3 HN^{12}C (a) and HN^{13}C (b) in Sag B2. Note gross difference in line profiles, both to same scale. Resolution = 1 MHz ($\approx 3.3 \text{ km s}^{-1}$).

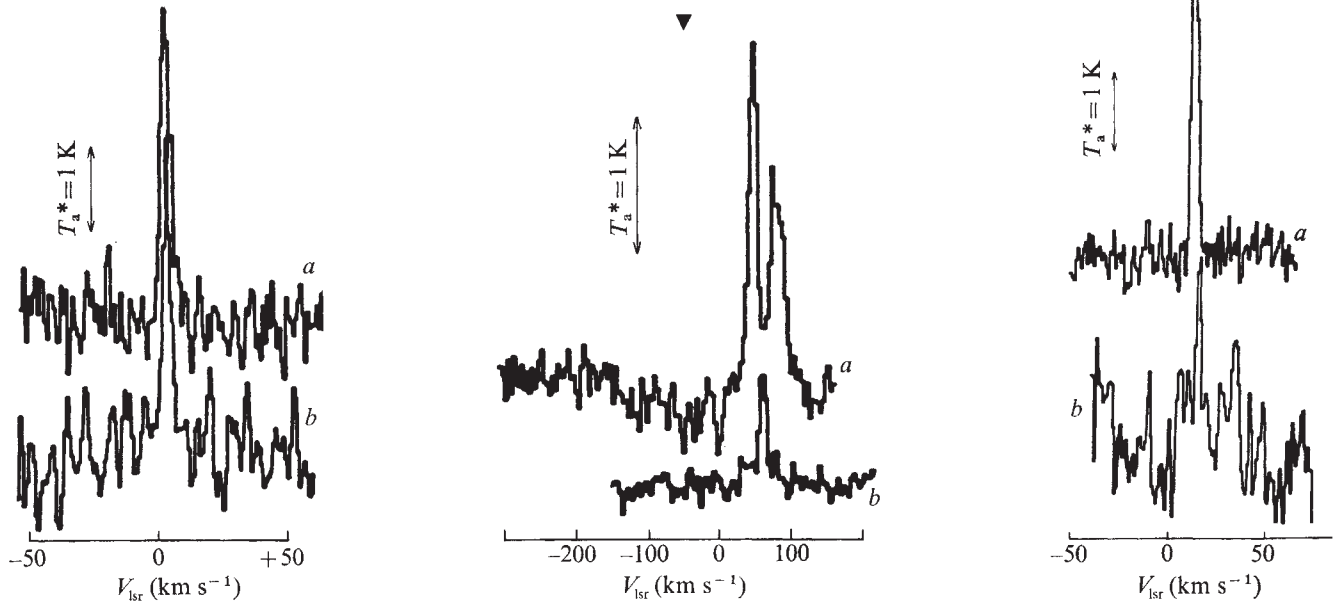


Fig. 2 HN^{12}C (a) and HN^{13}C ($\times 10$) (b) in DR21 (HCN peak). Note HN^{12}C line is significantly broader; resolution = 250 kHz ($\approx 0.83 \text{ km s}^{-1}$).

Fig. 4 HN^{12}C (a) and possibly HN^{13}C ($\times 10$) (b) in NGC2264. Resolution = 250 kHz ($\approx 0.83 \text{ km s}^{-1}$).

yielded the ^{13}C isotope transition frequency⁴. Immediately following this measurement, we searched for and detected the HN^{13}C $J=1\rightarrow 0$ rotational spectral line at 87,090.6 MHz in the galactic molecular source W51 (Fig. 1) on the NRAO 11-m telescope in March, 1976. Because of calibration problems with our receiver, we simultaneously observed the ^{12}C isotope so that relative abundance determinations might ultimately be made from our data. Appropriately, W51 was the source in which Snyder and Buhl first detected the $J=1\rightarrow 0$ transition of HN^{12}C . In addition, the HN^{13}C line was detected in DR21, Sag B2, and possibly also NGC2264 and IRC+10°216 (Figs 2–5). All of these sources also showed the HN^{12}C emission line. In addition, we detected HN^{12}C in W3 (Fig. 6), but were unable to detect the ^{13}C isotope there due to equipment failure. These results are summarised in Table 1.

We attempted to refine the HN^{13}C rest frequency in the narrow line source DR21. Assuming that HN^{13}C occurs at the common velocity of -3 km s^{-1} for this source position, our observations indicate a rest frequency of 87,090.71 (0.25) MHz. This compares favourably with our laboratory data and those of Creswell *et al.*⁵. Snyder and Hollis's⁶ rest frequency for HN^{12}C of 90,663.37 MHz indicates, however, a velocity of -5.25 km s^{-1} . This would necessitate a rest frequency of 87,091.36 MHz for HN^{13}C which is further from the laboratory data than one would like. Thus our data indicate the HN^{13}C frequency to be slightly higher in

frequency than our laboratory measurement. This is also more consistent with our data in W51 and Sag B2 (taken with lower resolution).

Our Sag B2 spectra (Fig. 3) are very interesting. The HN^{12}C spectrum displays a double peaked structure with a profound dip in the middle. A similar double structure has also been observed in N_2H^+ (ref. 7) and the $J=2-1$ transition of $^{12}\text{C}^{32}\text{S}$ (ref. 8) (we observed the $J=2-1$ of $^{13}\text{C}^{32}\text{S}$ to be a single broad feature centred on roughly 60 km s^{-1}). HN^{13}C , however, displays a very narrow feature almost exactly centred on the HN^{12}C dip, with a weak broad base underneath. The most straightforward interpretation of these data is that the HN^{12}C is optically thick and self absorbed while the HN^{13}C is not. The position of the narrow HN^{13}C peak is consistent with this interpretation, as is the broad base. The very narrow HN^{13}C feature is,

Fig. 5 HN^{12}C (a) and possibly HN^{13}C (b) in IRC+10°216. Note scales are unrelated. Resolution = 1 MHz ($\approx 3.3 \text{ km s}^{-1}$).

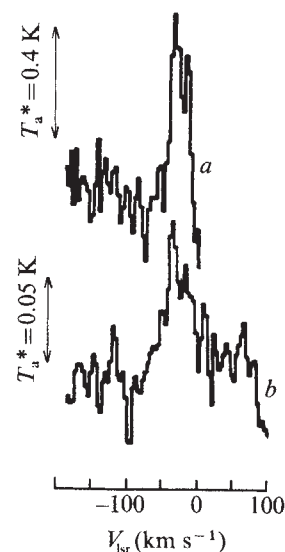


Table 1 Results of observations

Source name	RA (1950.0)	dec. (1950.0)	$T_a^* \text{ HN}^{12}\text{C}$ (K)	$T_a^* \text{ HN}^{13}\text{C}$ (K)
	(h min s)			
W51	19 21 23	+14° 24' 30''	4.8	0.18
DR21	20 37 10	42° 09' 00''	3.3	0.39
Sag B2	17 44 11	-28° 22' 30''	2.8 (complex)	0.95
NGC2264	6 38 28	9° 32' 06''	4.1	(0.27 ?)
IRC+10°216	9 45 15	13° 30' 40''	0.6	(0.11 ?)

T_a^* is antenna temperature divided by the beam efficiency corrected to outside the Earth's atmosphere.

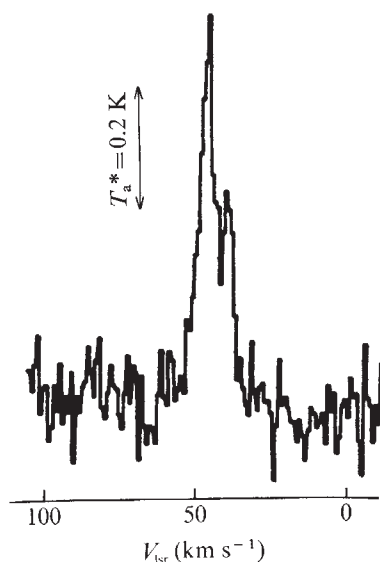


Fig. 6 HN^{13}C in W3. No data were obtained on HN^{13}C due to equipment problems. Resolution = 250 kHz ($\approx 0.83 \text{ km s}^{-1}$).

however, intriguing and we cannot help but be impressed with the possibility that the HN^{12}C and HN^{13}C are not spatially coexistent. Perhaps both conditions occur to some extent. One interpretation of the extremely narrow HN^{13}C feature is that it results from a localised region considerably enhanced in ^{13}C . This problem is certainly worthy of further study. Similar effects in the isotopes of HCN have previously been observed by R. A. Linke (private communication) and are currently under study by him (and coworkers).

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The second Z-propagation window

ELLIS^{1,2} investigated the condition in the ionosphere under which an upgoing ordinary radio wave is converted, through a coupling process, into an upgoing extraordinary wave in the Z mode. (Propagation in the Z mode requires the presence of both an ionised gas and a magnetic field; hence such waves cannot be received in free space.) This occurs for a wave incident

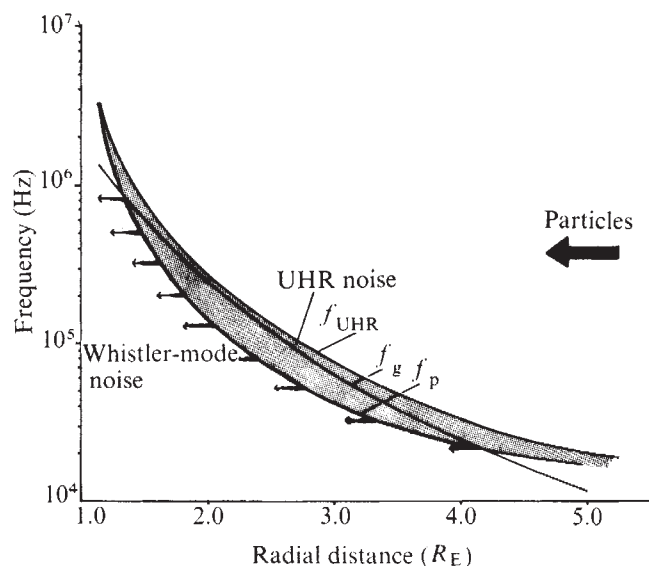
on the ionosphere from below with its wave normal close to a critical direction in the magnetic meridian plane. This direction is such that when the wave reaches the level where $X = 1$, which is normally a complete reflector for the ordinary wave, its wave-normal direction has become close to the magnetic field line direction. The symbols X and Y are as usually defined in magnetoionic theory (see Ratcliffe³). The level $X = 1$ becomes semi-transparent for a cone of angles centred on the critical direction. This cone is called the 'Ellis window'. The Z-mode waves are reflected at a higher level where $X = 1 + Y$. Here I wish to draw attention to a window which is relevant to the escape of Z-mode radiation produced naturally in the topside ionosphere.

It is well known from satellite observations that radio noise is produced by charged particles streaming through the magnetosphere and ionosphere (see Mosier *et al.*⁴ and references therein). One frequency range in which particularly intense noise is observed is that lying between the plasma frequency f_p and the upper hybrid resonance (UHR) $f_{\text{UHR}} = (f_p^2 + f_g^2)^{1/2}$ where f_g is the gyro-frequency; all frequencies refer to conditions local to the spacecraft. This is the frequency range where Z-mode radiation can attain very low phase velocities allowing interaction between waves and particles, and it is generally accepted that the intense noise is in the Z mode. Normally the noise cannot escape very far from the source region since it is reflected from the level where $X = 1 + Y$ and absorbed at the level where $X = (1 - Y^2)/(1 - Y^2 \cos^2 \theta)$ where θ is the wave-normal direction. If, however, the waves propagate past the level $X = 1$, which lies between the above two levels, with their wave-normal directions close to the magnetic field direction then the coupling process between Z mode and ordinary mode considered by Ellis becomes operative. If, in addition, $Y > 1$ at the level $X = 1$ then another propagation window appears involving coupling between Z-mode and whistler-mode waves, the latter being free to propagate to the ground.

The condition that $Y > 1$ at $X = 1$ is probably met at geomagnetic latitudes $\geq 70^\circ$ for altitudes $\geq 3,000 \text{ km}$, as is apparent from Fig. 1 which is based on our present limited knowledge of the plasma-density in this region⁵. The frequencies which could be coupled are seen to be $\sim 20 \text{ kHz} - 800 \text{ kHz}$.

It is significant that auroral hiss, which is broadband noise observed on the ground and on satellites in the geomagnetic

Fig. 1 Polar ionospheric model showing the expected radial variation of the plasma frequency f_p , the gyro frequency f_g , and the UHR frequency f_{UHR} . The region between f_p and f_{UHR} is where UHR noise from charged particle fluxes is expected. Whistler-mode noise appearing as a consequence of propagation through the second Z-mode window is shown shaded below the f_p curve.



latitude range 70° – 80° , covers a rather similar frequency range⁶. Therefore in any computation of hiss intensities it is important to consider the possible contribution from radiation which has passed through the second Z-propagation window mentioned here.

It is clear that the process is reversible, in that upgoing whistler waves with wave normals lying within a cone centred on a certain critical direction, can be coupled to Z radiation at $X = 1$ in the topside ionosphere. The Z radiation so produced will be absorbed at the level $X = (1 - Y^2)/(1 - Y^2 \cos^2 \theta)$ where the wave energy is transferred into heat resulting in an increase in the temperature of the local plasma. Thus it may be possible to consider this process as a means of modifying the topside ionosphere by using a high power, low frequency transmitter at high latitudes.

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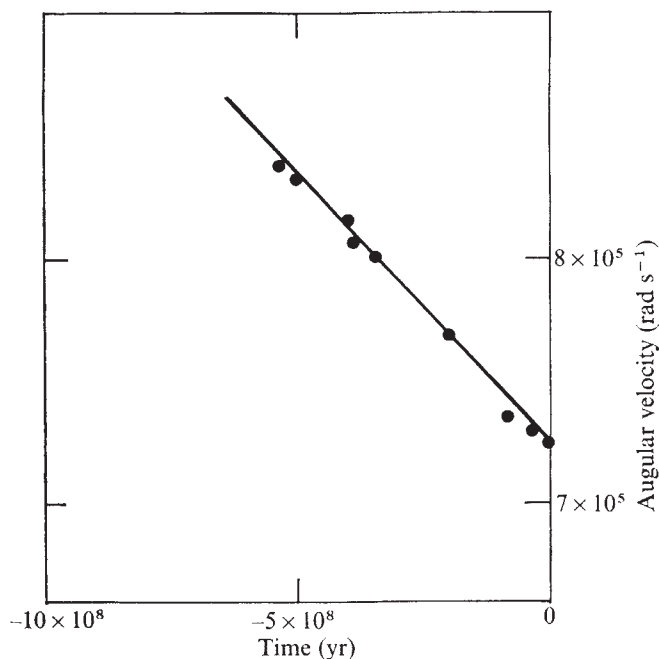


Fig. 1 The rotational velocity of the Earth derived from fossil corals during the past 540 Myr.

The total angular momentum of the Earth–Moon system is given by

$$P = C\Omega + mvr + c\omega$$

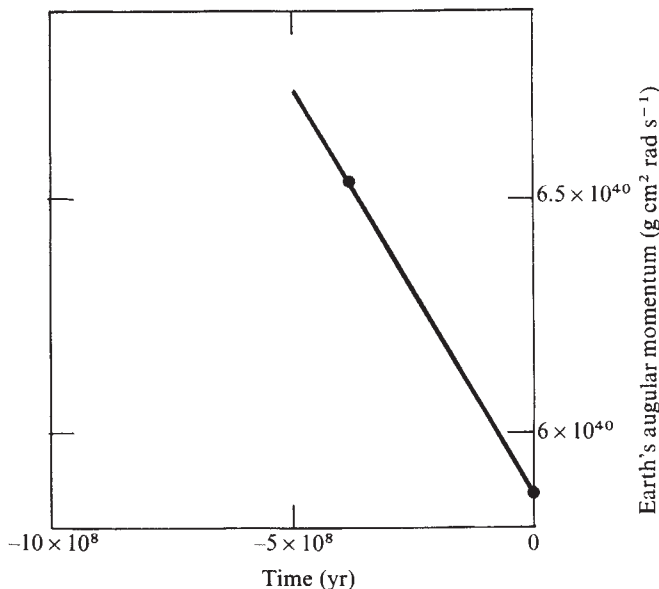
where m is the mass of the Moon, v is the linear velocity of the Moon in its orbit, r is the distance of the Moon, ω is the angular velocity of rotation of the Moon, and c is the moment of inertia of the Moon. As both $c \ll C$ and $\omega \ll \Omega$ we can ignore $c\omega$ so that

$$P \simeq P_E + P_M$$

where $P_E = C\Omega$ and $P_M = mvr = m(GMr)^{1/2}$ where M is the mass of the Earth.

As a closed system cannot lose angular momentum, the Moon must gain the angular momentum lost by the spinning Earth. If

Fig. 2 The decrease of the angular momentum of the Earth calculated by counting growth ridges in fossil corals.



Is the Earth expanding?

THERE have been many determinations of the acceleration of the Moon's mean longitude, mostly based on observations of occultations since 1680 and on records of ancient eclipses^{1–9}. All these results have been expressed in Ephemeris Time, based on the annual apparent motion of the Sun about the Earth. Since 1955 Atomic Time came into use based on the period of oscillation of the caesium atom which is, by definition, constant. A new analysis of lunar occultations observed from 1955 to 1974 using Atomic Time, by Van Flandern (unpublished) gives a value for the acceleration of the Moon's mean longitude differing from other determinations which used the Ephemeris Time scale. His new results differ from earlier work¹⁰. The data reveal a difference between Ephemeris Time and Atomic Time of 25 s a century. Van Flandern suggests that the most probable explanation is a decrease in the gravitational constant, G , by 8 parts in 10^{11} per yr. Such a decrease would mean that the Earth is expanding somewhat and there is some geological evidence supporting this. We tried to check this conclusion using a method for determining the lengthening of the day verified by Wells¹¹ in which the number of tiny lines of ridges on well preserved epitheca of corals in their annual growth increments are counted. We found no evidence for any expansion of the Earth in the past 5×10^8 yr.

More extensive results have been published by Panella *et al.*¹² giving the number of days in the year for different geological periods. Their oldest corals are from the Cambrian, 520 Myr ago. The Table of Panella *et al.* giving the number of days, T_E , in the sidereal year has been extended by us to derive the length of the day, D , in hours and the angular rotational velocity, Ω , of the Earth. By plotting the time dependence of Ω (Fig. 1) and by assuming a linear decrease, which seems fully justified, we find a value of the time derivative

$$\dot{\Omega} = (-6.88 \pm 0.05) \times 10^{-22} \text{ rad s}^{-2}$$

From this it follows that

$$\dot{\Omega}/\Omega = -(29.91 \pm 0.05) \times 10^{-11} \text{ yr}^{-1}$$

is in excellent agreement with ref. 9.

we define the present angular momentum of the Earth as $P_E = 1$, the present value for the Moon is $P_M = 4.964$, and $P = 5.964$. Therefore

$$P_E = 5.964 - 4.964(r/r_0)^{1/2} \quad (1)$$

where r_0 is the present distance of the Moon and we ignore the small variation of G .

Scrutton¹³ has studied other bands between the yearly expansions found on some fossil corals and he suggested that they might reflect monthly fluctuations in growth influenced by variations in the light of the Moon. Each band contained 30.6 d and the corals investigated lived ~ 380 Myr ago in the Middle Devonian. From his 30.6 d we can calculate the length of the synodic month in hours for that time by using the length of the day, D , found in ref. 12. By using the number of days in the year we can then calculate the length of the sidereal month in the Middle Devonian and with the third law of Kepler we can easily derive from it the lunar distance r at that time.

The value of P_E in the Middle Devonian can now be found by substituting the value of r in equation (1), and from its value 6.53×10^{40} g cm² rad s⁻¹, plotted in Fig. 2, we derive

$$\dot{P}_E = -1.76 \times 10^{40} \text{ per } 10^9 \text{ yr}$$

and

$$\dot{P}_E/P_E = (-30 \pm 3) \times 10^{-11} \text{ per yr}$$

As $P_E \approx 0.4MR^2\Omega$, where R is the radius of the Earth, we may write

$$\frac{\dot{P}_E}{P_E} = 2 \frac{\dot{R}}{R} + \frac{\dot{\Omega}}{\Omega}$$

The value of $\dot{\Omega}/\Omega$ derived from Fig. 1 is practically the same as the value of $\dot{P}_E/P_E$ found from Fig. 2. The conclusion seems inevitable that

$$\dot{R}/R \approx 0$$

so that the Earth has not expanded much during the past 5×10^8 yr.

An expansion of the Earth has already been pointed out by Holmes¹⁴ and by Wesson¹⁵ in a discussion of continental drift and seafloor spreading. If G is decreasing, the Earth should expand because of the decreased weight of the surface layers. This idea has been developed by Van Flandern (unpublished) in his discussion of the decrease of G . It would be very desirable that more extended counts on growth lines be made for fossil corals of different geological periods to check the points in Fig. 2.

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Test of the dipolar nature of the geomagnetic field throughout Phanerozoic time

THE morphology of the geomagnetic field throughout the Earth's history is of interest first, in a general way, to geomagnetists in terms of long term implications concerning the Earth's core, its thermal regime and the motions in it, and, second, in a very particular way, to palaeomagnetists since the geocentric dipole model is universally used in mapping observed field directions into palaeomagnetic poles. Runcorn^{1,2} has given strong reasons for supposing the mean geomagnetic field to be symmetrical about the Earth's spin axis. He has also argued³, on the basis of early palaeomagnetic data, that a dipolar field coupled with continental motion is preferable to higher order axial multipole fields. Here I discuss an observational test, based on the much larger body of palaeomagnetic data now available, to discriminate between a dipole field and those of higher order multipoles.

Attention is restricted to the angle of inclination, I , since absolute reference meridians are not available in palaeomagnetic studies. Suppose a large number of instantaneous measurements of $|I|$ are made at randomly distributed geographical sites. For any given geomagnetic field a definite frequency distribution of $|I|$ exists, and can be obtained by simply estimating the relative surface areas of the globe corresponding to any chosen set of $|I|$ classes. For instance, a dipole field is horizontal at the equator and has $|I| = 10^\circ$ at latitude 5.1° , whereas the field is vertical at the spin axis and has $|I| = 80^\circ$ at latitude 70.6° . The surface areas of these two zones imply that if sampling is sufficient and geographically random we expect the $0 \leq |I| \leq 10^\circ$ band to constitute 8.8% of the results and the $80^\circ \leq |I| \leq 90^\circ$ band to constitute only 5.7%. Figure 1 shows the dependence of inclination, $|I|$, on latitude, λ for the first four multipoles (dipole, quadrupole and so on) obtained from the relationship

$$\tan I_l = -(1+I)Pl/(\partial Pl/\partial \theta) \quad (1)$$

where Pl is the Legendre polynomial of degree l , and θ is the colatitude ($\theta = 90 - \lambda$). In Fig. 2 the corresponding frequency distributions of $|I|$ are given. The most notable features are the markedly low value in the range 80° – 90° and the high peak in the range 60° – 70° for the dipole curve.

Before comparing the palaeomagnetic data with the theoretical curves it is essential to enquire if they do, in fact, represent a random geographical sampling. Referred to the present latitude/longitude grid they clearly do not, because of uneven distribution of land and the existence of areas of relatively intense scientific effort. Throughout the past 500–600 Myr, however, considerable polar wander and/or continental drift has taken place^{4,5} and we proceed on the assumption that these have been sufficient to render the palaeomagnetic sampling random in a palaeogeographical sense.

Values of $|I|$ have been compiled from the list of Hicken *et al.*⁶, who feel that it is "approximately complete up to 1971". To minimise the effects of spatially and/or temporally concentrated sampling, unit weight has been given to each $10^\circ \times 10^\circ$ latitude/longitude square for each geological period from Cambrian to Tertiary inclusive (~ 600 to 2 Myr BP). Thus if, for example, N results are available from a certain latitude/longitude square for a particular geological period, each $|I|$ datum has been given a weight of $1/N$. Use of the geological periods is convenient and can be justified on the grounds that apparent polar wander takes place at about 0.2° Myr⁻¹ and that the periods represent about $50 (\pm 20)$ Myr, which implies typical within-period movement of about 10° . This is comparable with typical palaeomagnetic errors, and thus implies that a finer subdivision would be of little value.

Data from the Quaternary and the Precambrian were not included. The large body of data from the Quaternary would

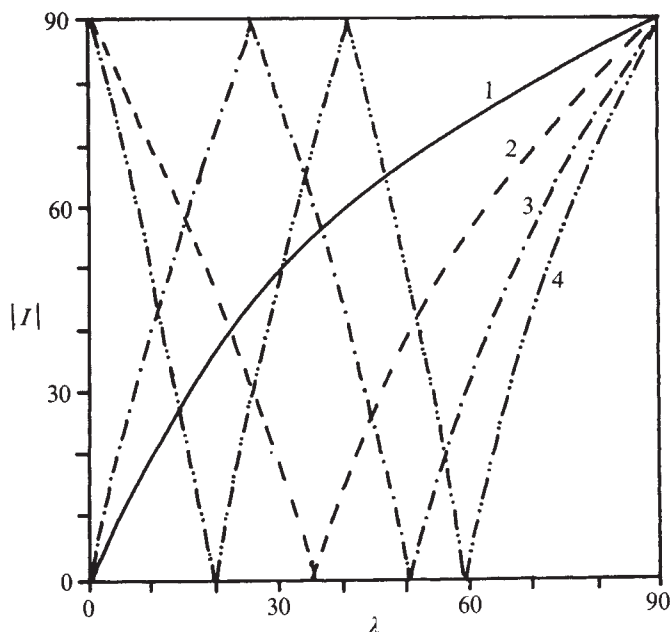
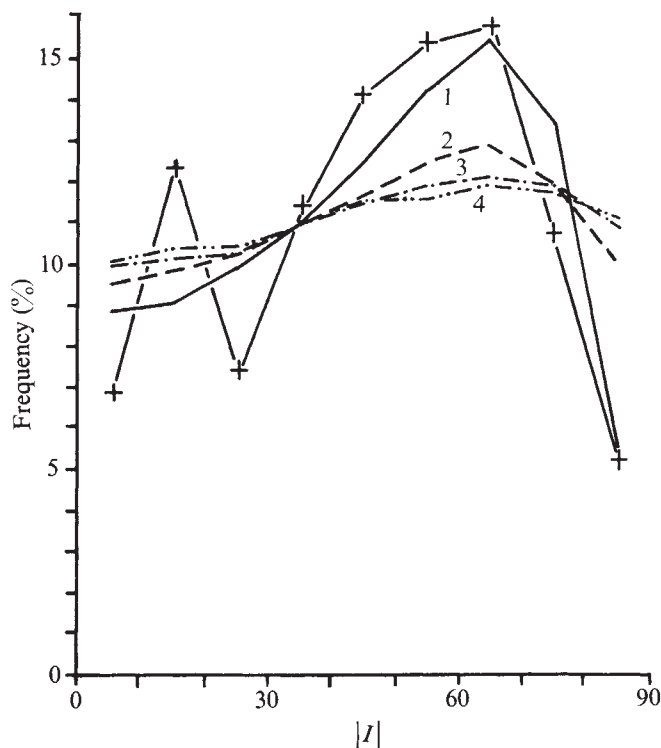


Fig. 1 Variation of angle of inclination, $|I|$, with latitude λ , for axial multipole fields. The numbers by the curves refer to the degree of the appropriate Legendre polynomial (1 = dipole; 2 = quadrupole and so on, see equation 1).

give undue weight to the past 2 Myr that it represents and, in any case, several authors^{7,8} have convincingly demonstrated the essentially dipolar nature of the field for this time interval. In the case of the Precambrian, the time involved is very long ($\sim 2 \times 10^9$ yr) but the data density is generally too low and is highly variable, and furthermore the assignment of accurate ages is often not feasible. For the time interval Cambrian to Tertiary the list of Hicken *et al.* yields a total of 1,271 inclination values, but the weighting procedure adopted reduces this to 430. The distribution of $|I|$ is shown in Fig. 2,

Fig. 2 Frequency polygons showing the distribution of $|I|$ for axial multipole fields. The curves, and the numbers by them, correspond to the curves of Fig. 1. +, Observations.



which indicates that the fit to the dipole curve is very reasonable, and is certainly favoured over the higher order multipoles. The main discrepancy, at low inclinations, probably reflects sampling inadequacies rather than any real feature since the total observations in the range $0-30^\circ$ is 26.8% which agrees closely with the expected value of 27.7%. Another factor adding noise preferentially to a dipole model at low inclinations could be the enhanced effects of secular variations at low latitudes⁹.

A χ^2 test can be used to compare the observations with the predicted distributions and yields $\chi^2 = 13.49$ for the dipole case. With eight degrees of freedom the critical value of χ^2 is 15.51 ($P = 0.05$) which indicates that we cannot assert that the two distributions differ. For $l \geq 2$ the same test strongly indicates that the observed distributions differ significantly from the expectations, χ^2 having values 27.98, 34.57, and 37.15 for $l = 2, 3$, and 4, respectively. Since palaeomagnetic errors are generally of the same magnitude as the class widths used here, it is perhaps worth while to calculate χ^2 for a coarser subdivision. Using three classes ($0-30^\circ$, $30-60^\circ$, and $60-90^\circ$) yields $\chi^2 = 2.37, 6.61, 8.88$, and 9.98 for $l = 1, 2, 3$, and 4 respectively, compared with the critical value of 5.99 (two degrees of freedom, $P = 0.05$). Again, no difference can be claimed for the dipole case.

I conclude that the frequency distribution of palaeomagnetic inclination angles implies that the geomagnetic field has been essentially dipolar throughout Phanerozoic time.

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Frequency of foreshocks

IN February 1975, the Chinese Seismological Bureau successfully predicted a magnitude 7.3 earthquake near Haicheng, Liaoning Province. From their reports, it is obvious that many methods such as radon count, abnormal animal behaviour, seismic velocity, and tilt and telluric current anomalies were considered in making the long term prediction¹. The short term prediction, however, was based primarily on foreshock activity^{2,3}. That foreshocks were important in the prediction raises the question of how common they are. We here analyse the frequency of foreshocks before shallow earthquakes with magnitudes greater than 7. Our findings show that 44% of all large shallow events in the world from 1950 to 1973 had foreshocks large enough to be teleseismically recorded, and that 21% of the large earthquakes that occurred in China between 1900 and 1949 had foreshocks large enough to be noted in local records.

We used two catalogues to cover the different periods of time. We searched the catalogue of Chinese earthquakes⁴ for the period 1900-49 for events preceding and near each earthquake with $M > 7.0$. Since the smallest magnitudes listed are about 4.5, and since they are listed only for the more recent portion of this period, the catalogue does not include many

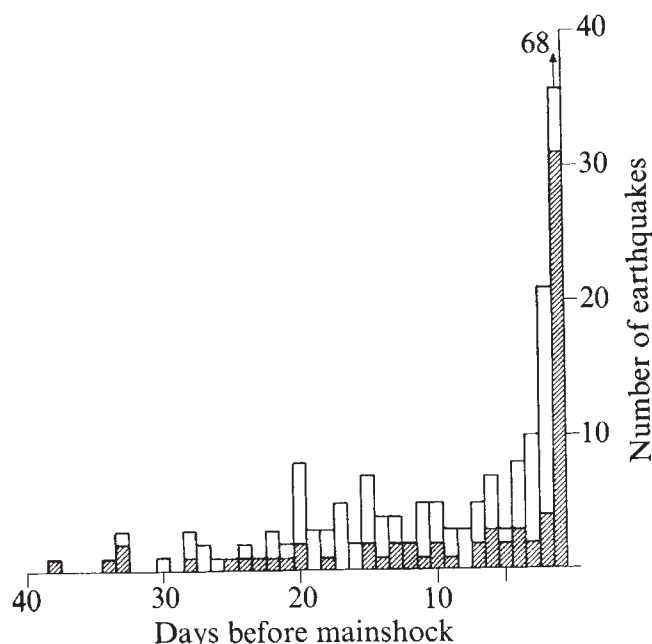


Fig. 1 Foreshock activity as a function of time before main shocks. For each day before main shocks the total number of foreshocks and the number of days before which the foreshock closest in time to the main shock (hatched area) occurred are shown.

small events that would have been recorded and located with present-day instrumentation. For the period 1950–73, we searched a catalogue of world events compiled by the National Oceanic and Atmospheric Administration (NOAA) for foreshocks before all events in the world with $M > 7.0$ (1950–64)⁸, and (1965–1973)⁹. The NOAA list includes all events located with teleseismic recordings by US Government agencies (Coast and Geodetic Survey, NOAA and Geological Survey) and by the Bureau Central International de Seismologie. Therefore, only events with $M \geq 4$ are included. The list is complete only for events with $M \geq 5$ (refs 7 and 8) for more recent years.

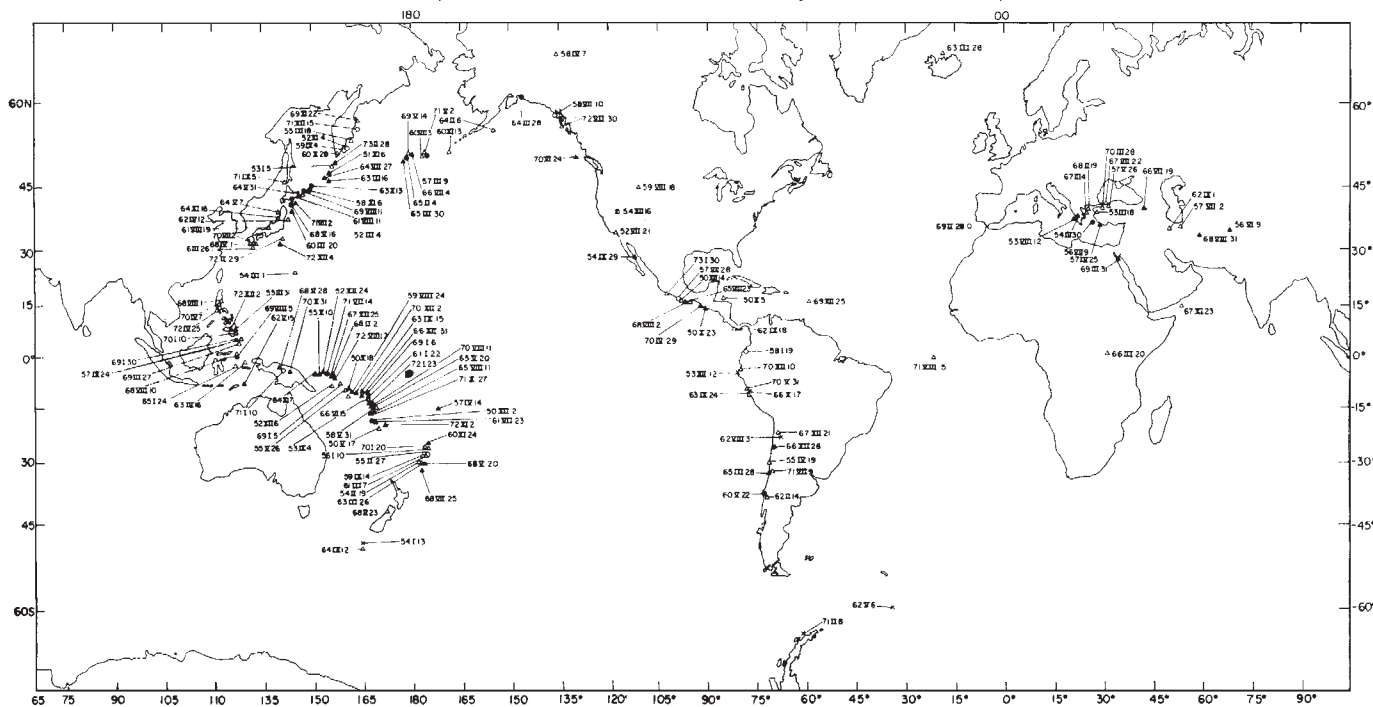
For some periods of time and some epicentral areas the location capability was particularly low, and no relatively large foreshocks or aftershocks were located. As we were interested in how many major earthquakes were preceded by foreshocks, we decided not to include events with two or less aftershocks listed in this catalogue. Thus, of the 246 events between 1950 and 1973 with $M > 7.0$, we used only 163. In fact, of 83 events with two or less aftershocks, 7 were preceded by at least one teleseismically located foreshock.

Because the source dimensions of earthquakes with $M > 7$ are of the order of 100 km, and, because of expected inaccuracies in epicentral determinations, only events located within 100 km of the main shocks were considered to be foreshocks, even though aftershocks often occurred at greater distances from them. To be classified as a foreshock an earthquake also had to occur within 40 d of the main event. By that criterion, however, 31 of the 72 cases of foreshock activity actually occurred within 24 h of the main shocks and 58 were within two weeks (Fig. 1).

Figures 2 and 3 show the geographical distribution of the main shocks studied, and also show which had teleseismically located foreshocks. Seventy-two of 163 major earthquakes between 1950 and 1973, or 44%, had at least one foreshock in the NOAA list. Of the earlier earthquakes in China, 10 of 48, or 21%, had foreshocks. The lower value for China probably reflects a less complete record for the period 1900–49 than for more recently. In fact, Imamura⁹ noted in 1937 that “big earthquakes” are preceded by foreshocks only 20% of the time, but anticipated that this percentage would increase as better instrumentation was installed.

It seems that even 44% is too low an estimate. Many earthquakes that had no foreshocks in the NOAA list (and thus not included in the 44%) are, in fact, known to have been preceded by foreshocks. For instance, Richter¹⁰ mentioned that a foreshock occurred within 24 h of both the 1952 Kern County, California, and the 1954 Fairview Park, Nevada, earthquakes. There were 35 earthquakes in the two days before the March 1966 earthquake in the Republic of the Congo¹¹, a week of foreshock activity preceding the 1965 earthquake in the Ceram Sea⁸, and 10 foreshocks within 24 h of the August 1, 1968 Luzon earthquake¹². Evidently, none of these foreshocks was

Fig. 2 Map showing earthquakes with $M \geq 7.8$ (circles) and $7.0 \leq M < 7.8$ (triangles) during 1950–1973, exclusive of China, for which 3 or more aftershocks were located. Events for which at least one foreshock (closed symbols) was located and no foreshocks (open symbols) were located are shown. $\times$, Events with less than 2 teleseismically located aftershocks, but with foreshocks.



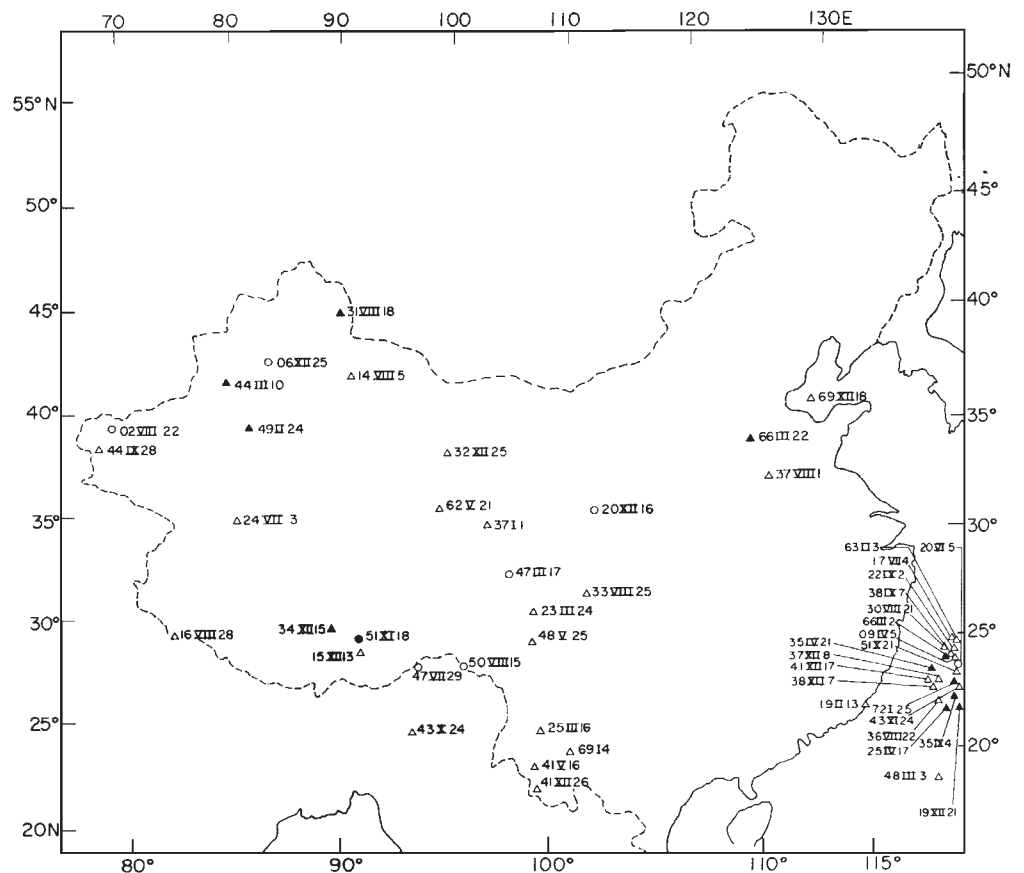


Fig. 3 Map of earthquakes in China. Symbols as in Fig. 2.

located teleseismically. Although foreshocks were the basis of the prediction of the 1975 Haicheng earthquake, none was teleseismically located by the US Geological Survey. We searched for field reports of the major events between 1950 and 1973, and found documentation of foreshocks in eight cases, for which no teleseismically located foreshocks were listed.

We sought possible correlations between the incidence of foreshocks and the magnitude or the tectonic setting of the earthquake, but no definite pattern emerged. Thirteen of the twenty-five great earthquakes ($M \geq 7.8$) had foreshocks, giving an incidence rate greater than 50%, but there are not enough data to make this conclusive.

That 44% of the earthquakes with $M \geq 7.0$ and at least three teleseismically located aftershocks were preceded by at least 1 teleseismically located foreshock suggests that foreshocks are a common occurrence. The Chinese have shown that they can be used to predict major earthquakes. The large number of locally recorded foreshocks preceding the 1965 Ceram Sea, 1966 Republic of Zaire, and 1968 Luzon earthquakes could have provided a data base comparable to that of Haicheng, and with adequate instrumentation may have opened the door to prediction. That is not to say that all earthquakes can be predicted by foreshock activity, as there do not yet seem to be definitive methods for discriminating foreshock activity from background seismicity. Not all earthquakes, in fact, seem to be preceded by foreshocks. The February 9, 1971, San Fernando earthquake ($M = 6.6$) is an example¹³. Moreover, it is not obvious that the magnitude of an impending earthquake could be predicted by foreshocks alone.

Nevertheless, with the growing evidence of short term premonitory slip before major earthquakes^{9,14-18}, the frequent occurrence of foreshocks may indicate that premonitory fault slip or accelerated deformation in the focal area is a ubiquitous phenomenon preceding all major earthquakes. Indeed, the rapid increase of seismic activity before major earthquakes

(Fig. 1) is similar to that of microfracturing observed in the laboratory¹⁹. Foreshock activity could well be the cause of many of the reports of anomalous animal behaviour. Together with aseismic deformation it could also be responsible for ground tilt, telluric current, magnetic field and well water level changes in the few days preceding major earthquakes. Thus it seems possible that all short term (and perhaps long term¹⁸) premonitory phenomena are a consequence of either seismic or aseismic slip before major earthquakes.

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Semiconductor liquid junction solar cells based on anodic sulphide films

ALTHOUGH good performance has been achieved with established solid state junction solar cells, their cost remains well above that required for large scale terrestrial applications. New concepts^{1,2} for photovoltaic energy conversion have been introduced which involve the junction between a semiconductor and an electrolyte. These schemes may lead to new possibilities of considerably reduced materials and fabrication costs, providing that many technical problems could be solved. We report here metal-semiconductor-liquid electrolyte junctions in which an n-type sulphide semiconductor is anodically formed *in situ* on its metal and, under photoexcitation, drives a sulphide-polysulphide redox couple when connected to a suitable cathode. The original contribution in this area was made by Gerischer² who demonstrated that the cell (single crystal *n*-CdS/Fe(CN)₆⁴⁻, Fe(CN)₆³⁻/SnO₂) has an initial conversion efficiency > 5% (ref. 3) for sunlight to electric power, in spite of the 2.4-eV band gap of CdS, which implies that wavelengths > 550 nm (the greater part of the incident solar photons) are ineffective.

In the Gerischer cell, photoexcitation of an *n*-CdS single crystal causes the transfer of holes to the reduced form of the redox couple and forms the anode of the solar battery. The circuit is completed through the transparent cathode of doped SnO₂ at which Fe(CN)₆³⁻ is reduced. As Gerischer has pointed out, the major problem in such a cell is that the reaction.



competes with the desired hole injection



and leads to rapid surface degradation. Such corrosion problems are inherent in most semiconductors with the exception of wide band gap materials such as *n*-TiO₂ used by Fujishima and Honda¹ for the photo-assisted oxidation of water.

Experience with CdS in this laboratory and elsewhere⁴ has shown that the problem of poor stability can be reduced, at the expense of lowering the driving potential and thus the conversion efficiency, by using an aqueous Na₂S-Na₂S_x redox couple. The overall anode reaction



competes more efficiently than reaction (2) with the corrosion path (1) because it proceeds at an electrode potential that is more negative by ~ 0.6 V. Additionally, any low level formation of sulphur can be prevented from covering the electrode by the dissolution reaction



We show that polycrystalline CdS, anodically formed in the presence of Na₂S, can replace single crystal CdS in solar cells. The preparation of such cells is simple and inexpensive. Other metals may be used to produce sulphide semiconductors with responses into the infrared which overlap better with the spectrum of sunlight.

Electrodes were made from materials of ≥ 99.99% purity using 10-mm sheet or cast rods of cadmium and bismuth cast from melted shot. The electrodes were defined by potting in epoxy resin. The current-voltage curves shown were obtained with rotating electrode disk configuration of active areas 0.429 cm² and 0.183 cm² for Cd and Bi, respectively. Counter-electrodes were either platinum or spectroscopic grade carbon rods. Spectra were obtained on stationary electrodes in vertical

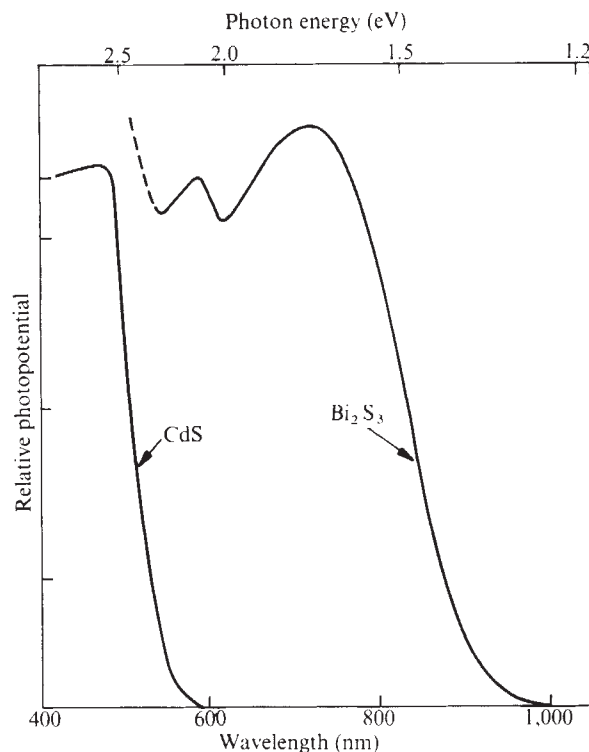


Fig. 1 Photoresponse spectra of the Cd/CdS electrode in 1F Na₂S and the Bi/Bi₂S₃ electrode in 1F Na₂S-0.05F S.

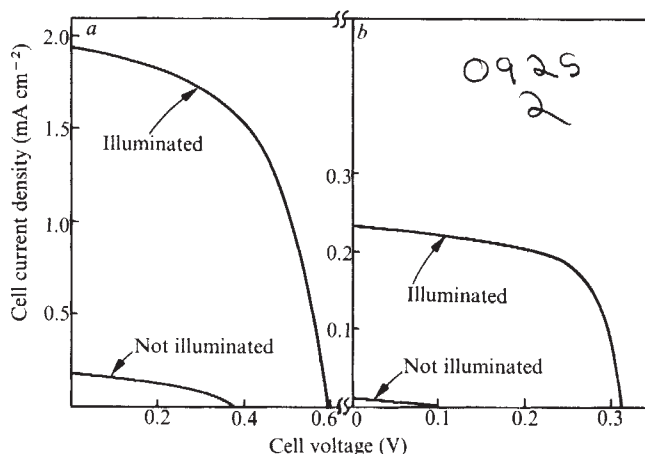
potted configurations. Potentials were measured against a standard calomel electrode (SCE).

Cells with Pyrex windows at the side (vertical specimens) or the bottom (rotating disk electrodes) were used for appropriate illumination of the sample. For present purposes no attempt was made to optimise the design or electrode placement for power efficiency.

Solutions were prepared from reagent Na₂S and flowers of sulphur and were filtered before use. Spectra of the yellow polysulphide solution were obtained on a Cary 14 spectrometer.

For photoresponse spectra, electrodes were illuminated by 400-Hz chopped, monochromatic radiation obtained with a 650-W tungsten (iodine) lamp and a 1-m Spex Czerny-Turner grating monochromator. The electrochemical response was processed with a lock-in amplifier referred to the chopper frequency. A Corning 2-58 filter, which cuts off wavelengths < 6,300 Å, was used to prevent harmonic response at the higher wavelengths. The photoresponse spectra were corrected for

Fig. 2 Current-voltage characteristics of the non-illuminated and illuminated cells (a) Cd/CdS/Na₂S-Na₂S_x/C and (b) Bi/Bi₂S₃/Na₂S-Na₂S_x/C with electrolyte concentrations 1F Na₂S and 0.05F S.



monochromator throughput, light source spectrum, and solution absorbance.

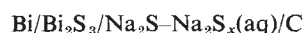
The cells were activated by anodising the metal (Cd or Bi) in the Na_2S_x electrolytic solution. Anodisation could be accomplished both galvanostatically and potentiostatically. Following anodisation, the electrodes develop an opaque layer of the sulphide which, as expected, is yellowish in the case of CdS and black in the case of Bi_2S_3 .

The electrochemical control circuitry used a conventional operational amplifier galvanostat-potentiostat. Photovoltaic cell operation was simulated by applying constant or linearly scanned currents in the spontaneous direction (Cd or Bi sulphides anodic) and then measuring the cell voltage (counter-electrode positive) and/or the potential of the photoelectrode against SCE which separated out any appreciable polarisation from the counterelectrode reaction. Photo-response spectra were obtained by chopping the incident beam and using as synchronously chopped output either (a) the potential relative to the counterelectrode or the SCE at open circuit or constant load current or (b) the current output when the photoelectrode was operated potentiostatically. The spectral response was found to be independent of the mode of operation or load.

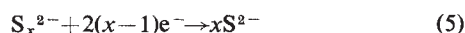
The two cells considered in this work are



and



The anode reaction of these cells is shown in equation (3). The cathode reaction is



As noted from the sum of equations (3) and (5), there is no net chemical change in the functioning cells.

Figure 1 shows the photoresponse spectra of the n-CdS and n- Bi_2S_3 surfaces formed by anodisation in 1F Na_2S and 1F $\text{Na}_2\text{S}-0.05\text{F S}$, respectively. As expected from the 2.4-eV band gap of CdS (ref. 5), the response of the electrode starts declining at 480 nm and becomes negligible at 580 nm. The band gap of Bi_2S_3 is $\sim 1.3-1.4$ eV (refs 6, 7) and the photoresponse is observed even at 900 nm, as expected. This response overlaps a substantial part of the solar spectrum after atmospheric filtering⁸.

The current-voltage curves of Cd or Bi disk electrodes paired with a carbon rod cathode in very low level light and with illumination are shown in Fig. 2. The curves of both CdS and Bi_2S_3 are reasonably close to being rectangular, indicating no dominating internal resistance losses. The changes in cell voltage and the changes in the Cd or Bi potentials measured against the reference electrode (SCE) are nearly identical, and the latter are not shown. Overpotentials attributable to the carbon cathode appear to be negligible with the large cathode/anode area ratio of these cells.

The open circuit voltage of the Cd/CdS/ $\text{Na}_2\text{S}-\text{Na}_2\text{S}_x/\text{C}$ solar cell under illumination is 0.59 V (see Fig. 2) or about half that reported by Gerischer² for the CdS (single crystal)/Fe(CN)₆³⁻/SnO₂ system. This difference results from the higher Fermi level (redox potential) of the Gerischer solution. Sacrifice of part of the working potential leads to a cell which does not deteriorate by oxidation of surface sulphide to sulphur during operation.

It is apparent that the technique can easily be extended to other sulphides or anodic films. Considerable work remains to be done to establish whether efficiency and long term stability can be brought to levels that would make such cells practical for terrestrial solar energy conversion.

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Opto-acoustic spectroscopy of products of continuous photolysis of nitromethane

THE photolysis of nitromethane has been carried out with both continuous (see ref. 1) and flash sources (see ref. 2), but for continuous photolysis no concurrent spectroscopy has been reported. This is in general true of most photolysis experiments, since the steady-state concentration of intermediates produced by continuous sources is usually insufficient to record spectra without depending on some matrix isolation procedure. We report here on gas phase continuous wave photolysis experiments using opto-acoustic detection of the energy absorbed by intermediates from a continuous wave (CW) tunable dye laser³. This allowed the detection of intermediate concentrations of $\sim 10^{13}$ mol cm⁻³. Opto-acoustic detection was discovered by Bell⁴ nearly a century ago, but was recently revived as a means of monitoring low concentrations of pollutant molecules^{5,6}. In our experiments the intermediates act as the 'pollutant' molecules to be detected.

The reaction vessel was a 5-cm long block of stainless steel through which a channel 0.5 cm $\times$ 1.0 cm had been cut. This channel was connected to a sidearm containing a sensitive microphone and to a small reservoir containing the sample. Two quartz windows sealed the ends of the channel. The unchopped and unfiltered output from a 250-W medium pressure Hg lamp was focused down to pass through the channel from one end. Tunable CW dye laser radiation, chopped at ~ 1.4 kHz, passed through the cell in the opposite direction. If the incoming chopped radiation is absorbed by a constituent in the cell that part of the absorbed energy degraded to heat will, in a constant volume enclosure, set up a modulated pressure wave at 1.4 kHz detected by the microphone.

Previous experiments² on pure nitromethane yielded evidence of nitroxyl and formaldehyde production together with OH and CN radicals. Neither nitrogen dioxide nor nitrosomethane were detected in these conditions, whereas they had been detected in the presence of excess nitrogen. Since the primary process is most probably



NO_2 at least should be formed in the photolysis of pure CH_3NO_2 , and indeed was detected by electron paramagnetic resonance (EPR) measurements⁷.

Figure 1a shows the opto-acoustic spectrum between 580 and 610 nm obtained from a sample of pure nitromethane at a temperature of 50 °C (pressure ~ 90 mmHg) under continuous photolysis. It was confirmed that, before photolysis commenced, no signal in this wavelength region existed. The spectrum of Fig. 1a thus corresponds to a species created by the photolysis. Figure 1b shows the spectrum obtained on buffering the nitromethane with 275 mmHg of argon and Fig. 1c using 275 mmHg

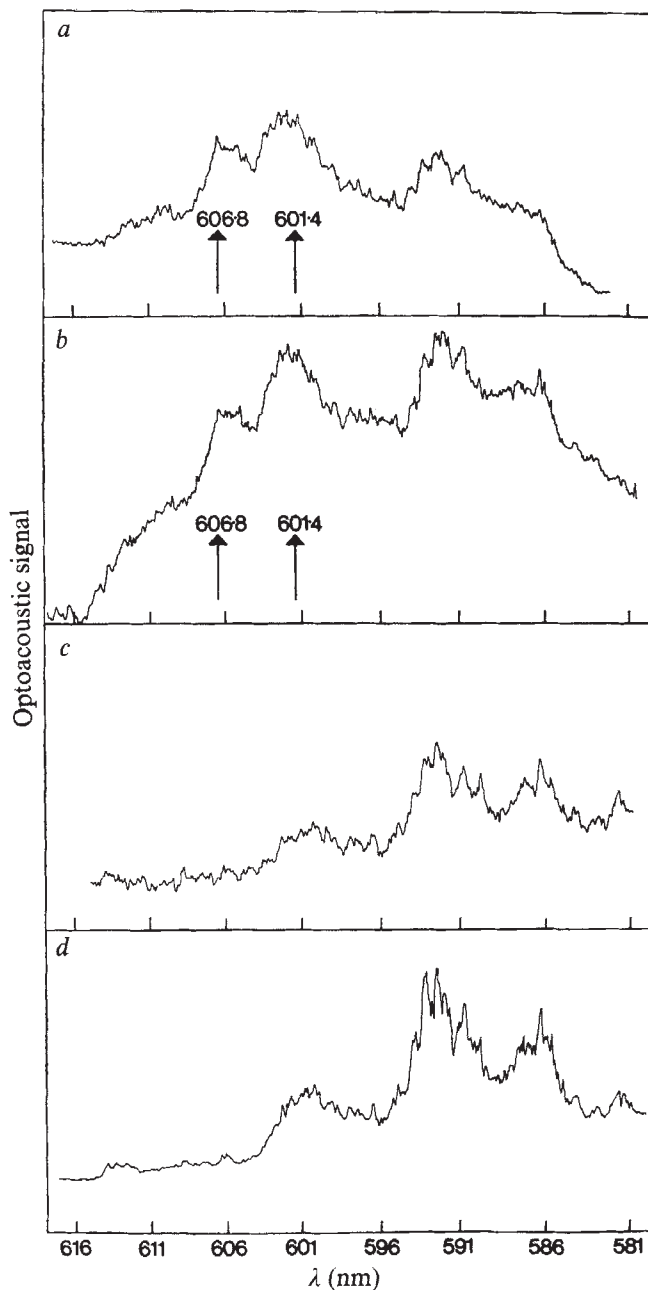
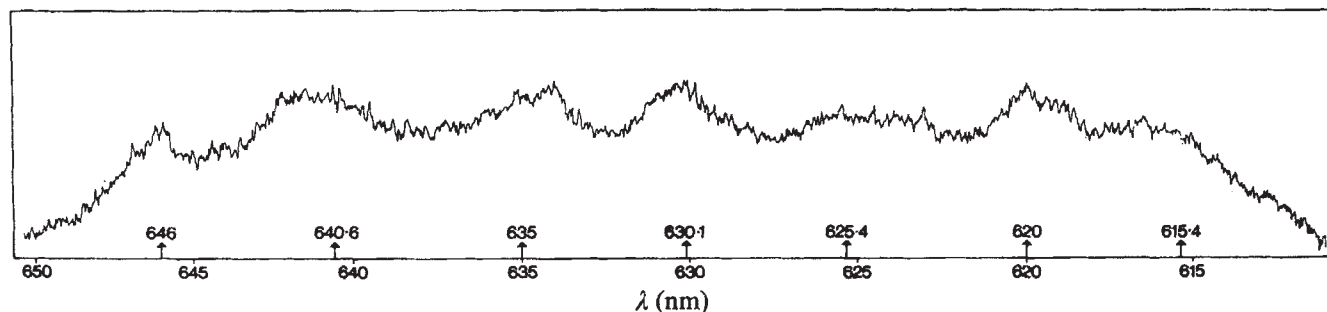


Fig. 1 Opto-acoustic spectra of CH_3NO_2 during continuous photolysis: a, pure CH_3NO_2 ; b, CH_3NO_2 in 275 mmHg argon; c, CH_3NO_2 in 275 mmHg oxygen; d, Opto-acoustic spectrum of 3×10^{-4} mmHg NO_2 in 300 mmHg N_2 . (The spectra are uncorrected for the smooth variation in dye laser power output over its tuning range.)

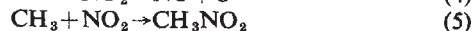
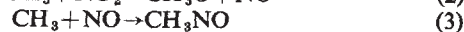
Fig. 2 Opto-acoustic spectrum (620–650 nm) of CH_3NO_2 in 275 mmHg of argon during continuous photolysis. Arrows mark the positions of band centres assigned to nitrosomethane (from Dixon and Kroto⁸).



of oxygen as a buffer. For comparison we show the spectrum of NO_2 taken in the same cell (but without the photolysis lamp) at $\sim 3 \times 10^{-4}$ mmHg in 300 mmHg of N_2 . It is evident from a comparison of the spectral features ~ 586 and 590 nm that we are recording the presence of NO_2 at levels $\sim 10^{-4}$ mmHg during the photolysis of nitromethane. The additional features at 607 and 610 nm disappear when oxygen is used as a buffer (Fig. 1c) leaving the pure NO_2 spectrum. The spectrum towards longer wavelengths of this second product is shown in Fig. 2, again from the photolysis of CH_3NO_2 in argon at 50°C . From the positions of the band centres by comparison with those given by Dixon and Kroto⁸ we can identify this product as nitrosomethane. It was confirmed in a separate experiment that NO_2 absorption in this region and at the concentrations inferred from Fig. 1c was not contributing $>10\%$ to the observed signal. In addition, its spectrum is markedly different to that of the nitrosomethane. We conclude that the spectrum of Fig. 2 is that of CH_3NO and that the features on the long wavelength end of the NO_2 spectrum of Fig. 1a and b come from the tail of CH_3NO absorption.

The build up of both CH_3NO and NO_2 on initial exposure took the same time of ~ 10 s. Similarly, the decay, a non-exponential with a fast initial fall changing gradually to give a long tail lasting ~ 20 min, was the same for both products. This decay behaviour was very different when O_2 was used as a buffer. In this case the NO_2 signal initially increased by $\sim 20\%$ and then followed a slower decay of up to ~ 30 min.

These results are consistent with the following set of secondary reactions following the primary split 1.



It is clear that in our relatively cool photolysis conditions and low intermediate concentrations (2) and (4) produce enough NO to yield measurable quantities of CH_3NO , whereas in the flash photolysis of Napier and Norrish² added amounts of NO were required before CH_3NO was detected. The alternative reaction²



is presumably less efficient than reaction (3). (We did not observe nitroxyl formation but, since its spectrum⁹ would be partially obscured by nitrosomethane, we do not rule out its formation.)

Reaction (6) (ref. 10) accounts for the identical bimolecular decay nature of NO_2 and CH_3NO although at some stage reaction (7) and the formation of nitrosomethane dimers become important². The change in decay behaviour of the NO_2 in the

O₂ mixtures, that is, without CH₃NO formed, is strong evidence for the influence of reaction (6). With O₂, CH₃NO formation is quenched, presumably by



or similar processes, and



and the decay of NO₂ governed solely by reaction (7). The rise in the NO₂ signal on removing the photolysing source probably arises from recombination of excess NO with oxygen.

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New light on the skull anatomy and systematic position of *Oviraptor*

Oviraptor philoceratops Osborn, 1924—a plunderer allegedly fond of ceratopsian eggs—is an Upper Cretaceous vertebrate assigned to the theropod dinosaurs¹. The holotype, comprising a compressed skull with mandibles, several cervical vertebrae, fragmentary shoulder girdle and forelimbs, was found in the Djadokhta Formation (?Santonian), Gobi Desert, Mongolia. For many years this was the only specimen known and its poor preservation showed only that *Oviraptor* was a toothless bipedal animal, with a lightly built skull, unusually short and deep. The lack of teeth and the structure of the manus were the main arguments for assigning it to the family Ornithomimidae^{1,2}.

In 1970 and 1971 the Polish-Mongolian palaeontological expeditions to Mongolia found two skulls (Fig. 1) of *Oviraptor* sp. with a few postcranial fragments in the Khermeen Tsav Formation (?Middle Campanian) as well as a fragmentary skull in the Nemegt Formation (?Upper Campanian—? Lower Maastrichtian), all in the Gobi Desert³. This material is housed in the Zaklad Paleozoologii (ZPAL) in Warsaw and I am now studying it. Five skeletons with skulls of *Oviraptor* sp. were found in 1972 by the Soviet-Mongolian palaeontological expeditions in the Khermeen Tsav formation⁴. This latter material is being studied by Dr R. Barsbold (Institute of Geological Sciences, Ulan Bator). All the specimens found recently belong to a new species. As the material being investigated by Barsbold is more complete than that at my disposal, I leave him to name and diagnose the new species, referring here to the forms housed in Warsaw as *Oviraptor* sp.

In the light of this new material the systematic assignment of *Oviraptor* may be questioned. The structure of the palate (Fig. 1e, f) is completely different from the palate of all other known dinosaurs. Its most striking feature is the form of the pterygoids. They are massive bars, with their lateral portions turned strongly ventrad. The ectopterygoids,

which in all other dinosaurs are directed transversely, joining the pterygoids with the jugal arches, lie parasagittally in *Oviraptor* and join the pterygoids with the maxillae, medial to the anterior roots of the jugal arches. Such a pterygoid-ectopterygoid structure is highly convergent with the dicynodont reptiles. The same may be said of the anterior palatal region formed of the premaxillae and maxillae, which bears a pair of ridges along its medial portion. Each maxilla in *Oviraptor* sp. produces a 'tooth' posteriorly, which is directed ventrally (Fig. 1a, b) and lies very close to its fellow. These 'teeth' are buttressed posteriorly by the thickened vomer. They do not bear any trace of wear and were therefore evidently covered in by a horny or soft tissue. The position of the palatine bones in *Oviraptor* is also different from that in other dinosaurs. The medial wings of the palatines border the choanae posteriorly, but laterally they form the ventral links between the maxillae and the ectopterygoids in a manner never seen in other dinosaurs.

Another unusual feature in a dinosaur is the very strong pneumatisation of the skull bones, especially those of the roof and snout. The nasals, in addition to being pneumatised as a whole, each bear a large air chamber connected with the external nares.

The endocranial cavity is well exposed on the two skulls I have studied, and is unusually large for a dinosaur, relative to the size of the skull. The approximate length of the cavity of the skull ZPAL No. Mg-1/95, measured from the foramen magnum to the dorsal border of the exit of the optic nerve, is 40 mm; its height (at the level of the optic nerve) is 17 mm; and its maximal width (just in front of the optic nerve) is 27 mm. The length of the skull is only 98 mm. The rapid broadening of the endocranial cast in front of the optic lobes is very striking, and corresponds to the position of the cerebral hemispheres, its total width almost double that of the diencephalon. The olfactory tracts must have been very short, as judged from the preserved portion of the endocranial cavity and from the close position of the external nares. Such a structure in the olfactory part of the brain seems to be non-dinosaurian and even non-reptilian (pterosaurs excluded).

The structure of the mandible of *Oviraptor* is also very different from that of other dinosaurs, and it was investigated in detail using the new material housed in Warsaw. The *Oviraptor* mandible (Fig. 1g, h) is basically similar to those described as *Caenagnathus collinsi* Sternberg, 1940 (ref. 5) and *C. sternbergi* Cracraft, 1971 (ref. 6) and assigned to the birds. The mandibles of *Caenagnathus* and *Oviraptor* have a similar shape, the same relationships between the bones and an identical articular region, consisting of a low ridge directed anteroposteriorly. They differ mainly in their proportions. Cracraft⁶ noticed that the jaw of *Caenagnathus* is structurally convergent with that of the dicynodonts; and this is in perfect agreement with my study of the skull of *Oviraptor*. *Oviraptor* and *Caenagnathus* should thus be assigned to the same family, Caenagnathidae Sternberg⁵.

Should the Caenagnathidae, however, be considered as birds? It is hardly possible that they are birds, taking into account the fact that known forelimbs of *O. philoceratops* do not show any distinct avian adaptations. In fact, the manus structure of this animal, with its three clawed digits subequal in length, conforms to that of the ornithomimids. The generally saurischian and theropod postcranial skeleton (confirmed by the Soviet-Mongolian expeditions; R. Barsbold, personal communication) may be evidence that, in spite of all the differences mentioned here, the Caenagnathidae should be regarded as theropod dinosaurs. Their skull structure is so peculiar, however, that long independent evolution of the group must be presumed. The caenagnathids may represent a Cretaceous continuation of the same dinosaur lineage that gave rise to the birds⁷. This could

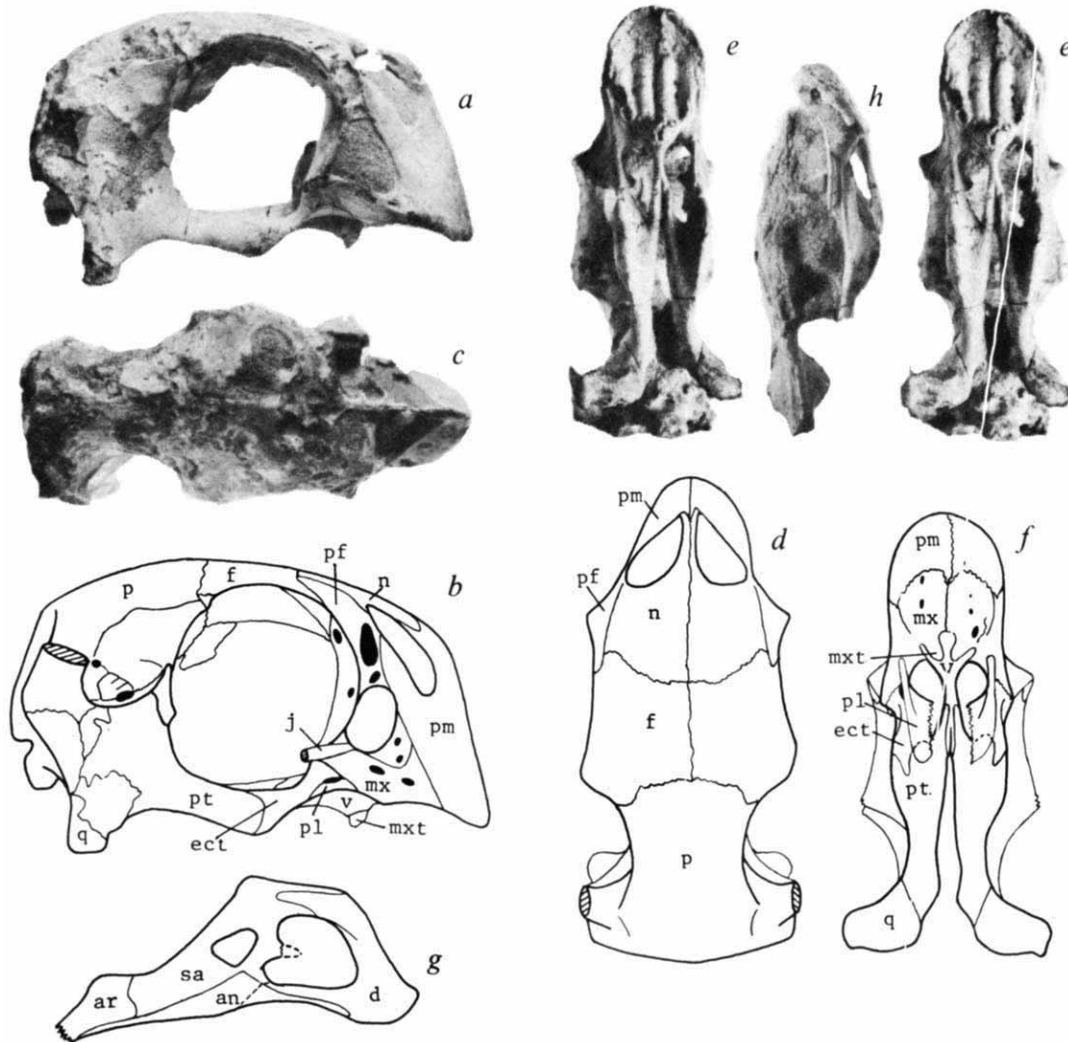


Fig. 1 *Oviraptor* sp. ZPAL No. MgD-I/95 skull lacking temporal and jugal arches: *a*, *b*, lateral view; *c*, *d*, top view; *e*, stereophotograph, palatal view; *f*, palatal view; *g*, mandible of the same specimen, lateral view; *h*, ventral view (approx. $\times 0.59$). an, Angular; ar, articular; d, dentary; ect, ectopterygoid; f, frontal; j, jugal; mx, maxilla; mxt, maxillary 'tooth'; n, nasal; p, parietal; pf, prefrontal; pl, palatine; pm, premaxilla; pt, pterygoid; q, quadrate; sa, surangular; v, vomer.

explain the presence of some para-avian characters in the caenagnathid skull, such as the separation of the pterygo-palatine complex from the jugal arch, the pneumatisation of the skull, the possible enlargement of the brain with the simultaneous shortening of the olfactory tracts and some structural characteristics of the mandibles. For these reasons it is not possible to assign the Caenagnathidae to any of the known theropod infraorders. A new supra-familial taxon will have to be established as soon as the new material of *Oviraptor* has been described.

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Hallucial tarsometatarsal joint in an Oligocene anthropoid *Aegyptopithecus zeuxis*

SINCE the grasping hallux is such a significant characteristic of the order Primates, its evolutionary history is of some importance to palaeoprimatologists. Although the origin and development of the grasping hallux in primates is usually hailed as a major "evolutionary event", very little factual data from the palaeontological record can be brought to bear in documenting this feature throughout primate evolution¹. Two fossil primates in which an hallucial metatarsal is known are *Notharctus* (an Eocene prosimian) and *Dryopithecus africanus* (a Miocene hominoid); hence the specimen discussed here is the earliest document of hallucial morphology in the anthropoids^{2,3}.

The earliest known postcranial remains of fossil anthropoids are from the Oligocene Jebel el Qatrani Formation of the Fayum badlands, Egypt. Most of the primate skeletal remains have been referred to the parapithecoid *Apidium phiomense*; one ulna has been referred to *Aegyptopithecus zeuxis*^{4,5}. Included among the various mammalian postcranial remains is an hallucial metatarsal of an anthropoid primate which was discovered by E. L. Simons at Yale

Quarry I in the Upper Fossil Wood zone. This is the quarry that has yielded mandibular and/or maxillary dental remains of several primate genera—*Parapithecus*, *Apidium*, *Aeolopithecus* and *Aegyptopithecus*⁸. Of these fossil primates, only *Aegyptopithecus* could have had a hallux as large as that suggested by the fossil hallucial metatarsal. For this reason the specimen is here referred to *Aegyptopithecus zeuxis*.

The specimen (YPM 25806) consists of the proximal two-thirds of a right hallucial metatarsal (Fig. 1). The bone is perfectly preserved except for a small sliver missing from its medial side. The proximal articular surface for the medial cuneiform is complete and undistorted. Its overall size suggests an animal whose body weight might be approximated by *Ateles belzebuth* (Fig. 2).

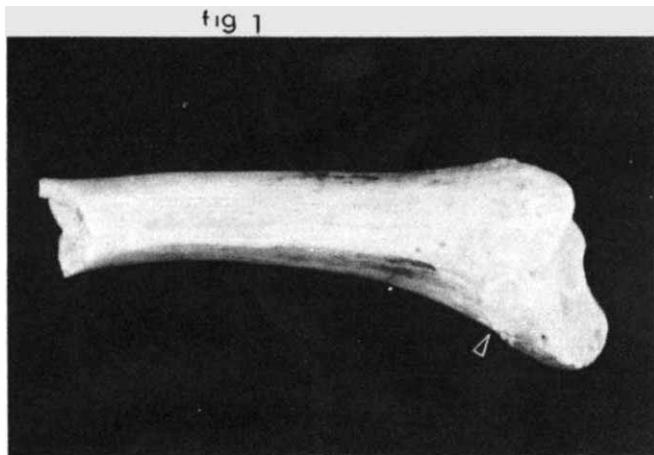


Fig. 1 Medial view of right hallucial metatarsal of *Aegyptopithecus zeuxis* (YPM 25806). Note prehallucial facet (arrow).

Much information concerning the morphology of the primitive anthropoid hallucial tarsometatarsal joint can be gathered through examination of the proximal articular surface of the hallucial metatarsal of *Aegyptopithecus zeuxis*. Of particular interest is a well defined, raised oval facet on the medioplantar aspect at the base of the metatarsal. This undoubtedly reflects the fact that a large prehallux bone participated in the hallucial tarsometatarsal joint. Thus, this joint consisted of three bones in primitive anthropoids, the first metatarsal, medial cuneiform and prehallux. This condition is unlike that of any extant Old World monkey or great ape; it is seen in most platyrrhines



Fig. 2 Comparison of hallucial metatarsals of several primates. a, *Galago crassicaudatus*; b, *Dryopithecus africanus*; c, *Cebus nigrivittatus*; d, *Hylobates hooloch*; e, *Ateles belzebuth*; f, YPM 25806. Note the attached prehallux in *Cebus* and *Ateles* and prehallucial facet on YPM 25806 (arrows).

and hylobatids⁷. On the lateral plantar aspect of the metatarsal base is a large, blunt, bony projection for the insertion of *M. peroneus longus* tendon. This muscle, an evertor of the tarsus and an adductor of the hallux, was obviously large in this primitive, arboreal fossil ape. In contrast, *Simopithecus*, a large, terrestrially adapted Pleistocene monkey, had a rather small peroneus longus muscle. This is evidenced by the relatively small peroneal tubercle and the small facet for the peroneus longus sesamoid on the cuboid⁸. The Fayum metatarsal also has a rather pronounced longitudinal curvature, indicative of strong flexor tendons⁹.

The shape of the joint surface itself, for articulation with the medial cuneiform, is essentially a concave ovoid with a nearly vertical axis around which abduction-adduction took place (Fig. 3). The ovoid widens in a plantar direction, thus producing an effect where the plantar surface of the metatarsal rotates around a greater arc than the dorsal surface. Because any point which traverses an arc along an ovoid surface will undergo conjunct rotation, this morphology is advantageous in any joint where opposition is desirable¹⁰. Thus, as the metatarsal moves over the articular surface of the medial cuneiform it undergoes conjunct rotation and comes more into opposition with the lateral four digits. The prehallux would have completed the joint surface at the medioplantar border. The dorsal and lateral margins of the metatarsal base show rugosities for tarso-metatarsal ligaments. During adduction of the hallux, the plantar surface of the bone would have been oriented to-

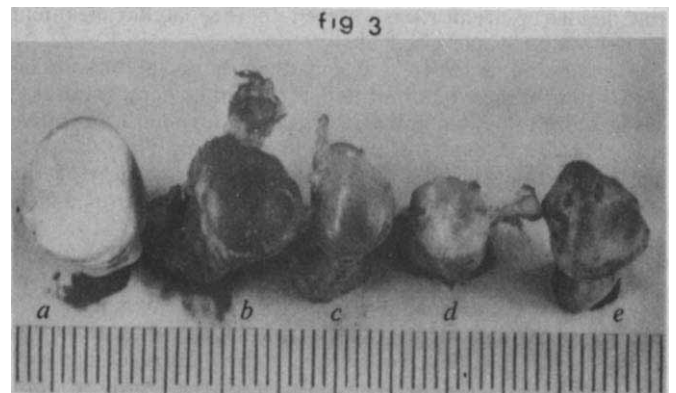


Fig. 3 View of proximal articular surface of hallucial metatarsals. a, *Dryopithecus africanus*; b, *Ateles belzebuth*; c, *Hylobates hooloch*; d, *Cebus nigrivittatus*; e, YPM 25806.

wards the lateral digits as in opposition. This is due to the morphology of the joint surface itself (see above) and to the fact that the shaft of the metatarsal shows a high degree of torsion in this direction. This torsion is evidenced by the fact that the greatest diameter of the shaft in cross section is nearly parallel to the vertical axis of the hallucial base.

Lewis has described the hallucial tarsometatarsal joint in anthropoids, and his predictions concerning the morphology of the hallucial tarsometatarsal joint in primitive anthropoids can be verified by the fossil evidence presented here⁷. He has shown that in primitive mammals (for example, *Didelphis*) an hallucial prolongation of the flexor tibialis muscle (the homologue of the *M. flexor digitorum longus* in the human) contains the prehallux. When the prehallux occurs in primates it forms an integral part of the hallucial tarsometatarsal joint and is invariably associated with the dual insertion of tibialis anterior into it and the medial cuneiform. The prehallux, judging from the shape of its articular facet on the hallucial metatarsal of *A. zeuxis*, had a sellar-shaped articular surface and formed part of a true synovial joint, as do most sesamoid bones in the body. Sellar-shaped surfaces are characteristic of joints which have opposition as a habitual movement¹⁰. The prehallux, or a cartilagenous remnant, is characteristic of

Ceboidea but not Ceropithecoidea. Among Hominoidea, it is seen in Hylobatidae but not Pongidae⁷.

It is perhaps interesting that a vestigial prehallux is occasionally found in modern man¹¹⁻¹³. The so-called "os paracuneiforme" was first described clinically in 1902 (ref. 14). Although surgeons were unsure of what this small accessory bone really represented, evidence now suggests that it is the vestige of the primitive anthropoid prehallux (my unpublished results). The "os paracuneiforme", like the prehallux, articulates with the medial cuneiform and is protected by an overlying bursa; as in anthropoids, the bone is partly incorporated in the substance of the tibialis anterior tendon. It is still unclear whether or not this is a true accessory bone or a sesamoid¹⁵. Alternatively, it might represent one of the unfused multiple ossification centres in the epiphysis of the metatarsal¹⁶. It seems most likely, however, that it is a sesamoid formed by pressure in the tendon of tibialis anterior as it passes over the medial cuneiform; indeed, such bones sometimes occur late in life in this position¹⁷. Experimental evidence on the patella, the largest sesamoid in the body, suggests that both phylogenetic and ontogenetic factors are responsible for the formation of sesamoid bones. For example, development of the patella has followed transplantation of limb bud fragments from chick embryos to the chorioallantoic membrane of other host embryos¹⁸. Conversely, patellar cartilage and bone which has been removed from young dogs will regenerate from young connective tissue cells which had not been destined as specific formers of bone if the tendon of the quadriceps femoris is sutured to the patellar ligament and the joint is exercised¹⁹.

The finding that *A. zeuxis* had an hallucial tarsometatarsal joint which incorporated a prehallux demonstrates a clear morphological similarity between this most primitive anthropoid and extant New World primates. This also supports recent evidence that the postcranial skeleton of the earliest anthropoids in general was more similar to extant platyrrhines than to catarrhines^{4,20}.

The morphological similarities between the Oligocene primates of the Fayum and extant platyrrhines have led to speculations regarding an African origin for the New World primates through rafting across the South Atlantic in the early Tertiary. Such a voyage seems hard to reconcile with the geophysical evidence suggesting that the South Atlantic was 3,000 km wide by Oligocene times²¹. Others have suggested that some primate family with a holarctic distribution in the Eocene (Adapidae?) might have given rise to both New and Old World anthropoids by the early Oligocene²². One point is becoming increasingly clear however—platyrrhines are the best models for depicting the earliest stages of anthropoid postcranial and locomotor evolution. Lewis' contention that the earliest anthropoids must have possessed an hallucial tarsometatarsal joint including a prehallux is undoubtedly correct. It is interesting that *Dryopithecus africanus*, a putative descendant of *A. zeuxis*, also apparently had a prehallux incorporated into its hallucial tarsometatarsal joint⁷. Thus there is nothing in the anatomy of the hallucial tarsometatarsal joint which would preclude *Aegyptopithecus* from being broadly ancestral to later dryopithecines and hylobatids.

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Control of intragroup aggression by male pigtail monkeys (*Macaca nemestrina*)

CERCOPITHECINE primates (*Macaca*, *Papio*, and *Cercocebus* spp.) typically live in cohesive groups containing many more adult females than adult males. Strong status hierarchies often exist within established groups, and one male in each group usually exerts an especially powerful influence on the behaviour of other group members. This individual commonly places himself between the group and any potential external threat and frequently interferes in intragroup aggressive encounters by attacking or threatening to attack one of the participants in the altercation¹. An animal displaying this pattern of behaviour has been referred to as a "control animal" or as fulfilling a "control role"².

While the existence of such a control role has been generally accepted, most descriptions of this behaviour have not measured the magnitude of the influence of male presence on the inhibition of violence among females. Cessation of aggression contiguous with attacks or threats by males has provided the bulk of evidence for control role behaviour. In one experimental study, however, removal of the control male from a group of pigtail macaques (*M. nemestrina*) resulted in increased aggression among other group members and his reinstatement in the group reduced aggression to preseparation level³. The removal and reinstatement of other group members did not influence intragroup aggression. Unfortunately that experiment used only one group of monkeys under one set of housing conditions, a situation hardly sufficient to establish the generality of the phenomenon.

Because we had access to a large number of groups of *M. nemestrina* and were concerned about the social mechanisms involved in the control of aggression in captive groups of non-human primates, we felt obliged to test the generality of the effect of mere presence of males on intragroup aggression. Six groups, each containing one adult male and an average of 8.5 adult females and 1.1 infants were studied. Each group was housed in identical spatial conditions (2.2 × 3.1 × 2.8 m), but the conditions differed considerably from those of the previously cited experiment. Each group was tested according to a sequence of three phases (each 20 min long): a pretest with all group members present to establish basal aggression rates; an experimental test immediately following removal of the male from each group; and a post-test immediately following reintroduction of the male into the group. An experienced observer recorded absolute frequencies of agonistic interactions of group members, noting the sex of the perpetrator and recipient of each act. The following activities were recorded: contact aggression (grab, push, hit, and bite); non-contact aggression (chase, open-mouth "threat", and "bark" vocalisation); and submission (grimace, "screech" vocalisation, and crouch).

Table 1 Incidence per capita of agonistic interactions among females*

	Group I (N = 9)			Group II (N = 9)			Group III (N = 12)			Group IV (N = 6)			Group V (N = 9)			Group VI (N = 6)		
Behaviour	PR	TE	PO	PR	TE	PO	PR	TE	PO	PR	TE	PO	PR	TE	PO	PR	TE	PO
Grab	0.22	1.67	0.11	0.11	1.44	0.11	0.08	0.75	0.00	0.50	1.17	0.17	0.11	0.67	0.22	0.33	1.67	0.00
Push	0.00	0.11	0.00	0.11	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00
Hit	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.17	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bite	0.00	1.00	0.00	0.00	0.78	0.00	0.00	0.42	0.08	0.00	0.67	0.17	0.00	0.00	0.00	0.00	1.00	0.00
Contact																		
Aggression	0.22	2.78	0.11	0.22	2.33	0.11	0.08	1.25	0.08	0.67	2.33	0.33	0.11	0.78	0.22	0.33	2.67	0.00
Chase	0.00	0.56	0.00	0.00	0.67	0.00	0.08	1.50	0.00	0.00	0.33	0.33	0.00	0.33	0.22	0.00	1.50	0.00
Threat	1.22	4.78	1.11	0.33	3.11	0.11	0.08	1.42	0.50	1.67	6.83	1.00	1.11	4.00	0.56	0.50	1.67	0.00
Bark	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Noncontact																		
Aggression	1.22	5.34	1.11	0.33	3.78	0.11	0.16	2.92	0.50	1.67	7.16	1.33	1.11	4.33	0.78	0.50	3.17	0.00
Grimace	0.00	0.22	0.00	0.00	0.44	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00
Screech	0.00	0.44	0.00	0.00	0.56	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00
Crouch	0.00	0.44	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Submission	0.00	1.10	0.00	0.11	1.00	0.00	0.00	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00
Total																		
Agonistic	1.44	9.44	1.22	0.66	7.11	0.22	0.24	4.51	0.58	2.34	8.99	1.67	1.22	5.11	1.00	0.83	6.34	0.00

*PR, pretest; TE, test; PO, post-test.

Per capita incidence of agonistic interactions among adult female *M. nemestrina* during pretest (PR), test (TE), and post-test (PO). One adult male was present in each group during the pretest and post-test and no male was present during the test phase.

As indicated in Table 1, more agonistic behaviour occurred during the absence of males than during either session in which males were present (Wilcoxon $T=0$, $P<0.05$). More than seven times as much contact aggression (primarily grab and bite) was observed when males were absent as was seen during either phase when males were present (Wilcoxon $T=0$, $P<0.05$) (see Fig. 1), and non-contact aggression (chase and "threat") followed a similar pattern with more than five times as much occurring during the period of male absence as during either of the other phases (Wilcoxon $T=0$, $P<0.05$). Submissive behaviour was not observed in all groups but when it was seen it was almost exclusively during the period of male absence.

The results of this study leave little doubt about the inhibitory influence of male presence on aggression among female *M. nemestrina*, although the brief periods of removal did not rule out the possibility that the response might have been temporary. Surveys of aggressive behaviour in 118 groups containing no males, one male, or more than one male⁴⁻⁸ have, however, convinced us that the violence precipitated by the removal of males from groups was not a temporary response. Significantly more aggression occurred

in groups that contained no males than in groups containing one or more males, but the presence of more than one male did not reduce aggression any more than the presence of one male. Another study (Swenson, Bartlett, and Sackett, unpublished) demonstrated that the effect was not simply due to removal of an animal from the group, because removal of high and low ranking females in similar conditions did not produce consistent changes in intragroup aggression. We are not arguing that females cannot or do not ever fulfil control role functions, but it seems clear from the available evidence that females do not typically assume the control role if one or more males are present. If females assume control role functions in the absence of males they apparently do not succeed in inhibiting aggression among other females as effectively as do males.

The control of aggression among females by males is apparently not unique to *M. nemestrina*, although little information on other species is available. When the dominant male was removed from a group of crab-eating macaques (*M. fascicularis*) for one night aggression was so severe that three adult females died of injuries (S. B. Christopher, unpublished). It is abundantly clear that removal of control animals from non-human primate groups should only be done with great care, and that there is potential danger in housing cercopithecine primates in all-female groups.

In free-ranging conditions the leader male spends most of his time near the centre of the group. If his primary role were protection of the group from sources of external threat he might instead be expected to maintain a peripheral position. Yet it seems that he has little choice in the matter for the females follow and cluster about him. Could it be that they do so for protection from each other?

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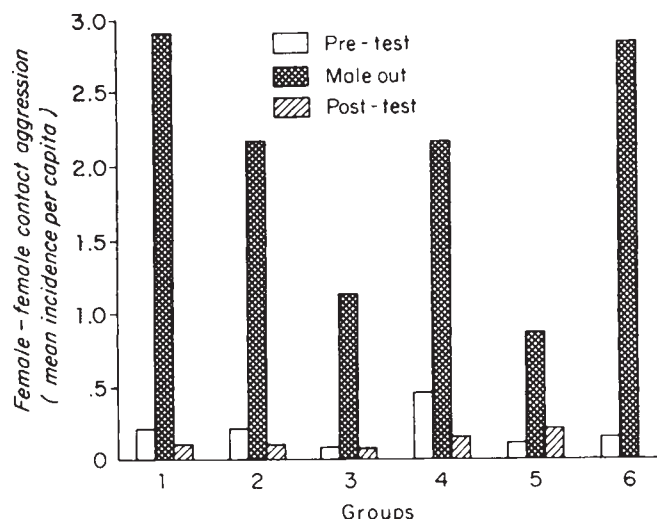


Fig. 1 Removal of adult males from groups of *M. nemestrina* resulted in dramatic increases in contact aggression among adult females. Reintroduction of males reduced female-female aggression to preseparation levels.

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Social and reproductive correlates of parasite ova emissions by baboons

In several species of rodents, the social and reproductive condition of a host individual has been shown to affect its susceptibility to intestinal parasites and the maturation rate of the parasites once established^{1,2}. The relationship between social and reproductive condition of host individuals and most aspects of parasite development and transmission, including ova emissions, has not previously been examined for any non-human primate species. Information on parasite ova emissions by non-human primates is important for understanding the life-cycle of the parasites and for understanding the spread of parasites both within and between social groups and to individuals of other species, including humans and domestic stock³.

In 1974, as part of an ongoing longitudinal study of free-living yellow baboons (*Papio cynocephalus*) in the Amboseli National Park, Kenya, we collected 100 faecal samples from the 29 adult and subadult members of a single well studied group⁴. Intestinal parasite ova were recovered from these samples by zinc sulphate flotation⁵ and number of ova per g of faeces was analysed in relation to dominance rank and reproductive condition of host. Egg recovery and counting was done "blind"; samples were identified only by a sequence number and not by name or other information concerning the host. Two nematode genera, *Trichuris* and *Trichostrongylus* accounted for 89% ($N = 2,119$) of all parasite ova recovered in the samples. The distribution of number of ova per g of faeces for all samples combined was positively skewed and roughly Poisson in shape; thus, significance testing was carried out with the variance ratio⁶.

Figure 1 shows that among adult and subadult males, high ranking individuals had higher egg emissions than did more subordinate individuals. All differences in mean egg counts between adjacent dominance rank classes were statistically significant ($P < 0.01$ for all comparisons); the Spearman rank correlation coefficient between mean egg count and dominance rank in adult and subadult males was 0.67 ($P < 0.05$). Subadult males generally had lower egg counts than did older, fully adult males, and ranks 13-16 were occupied exclusively by subadults. But, examination of the mean egg counts for

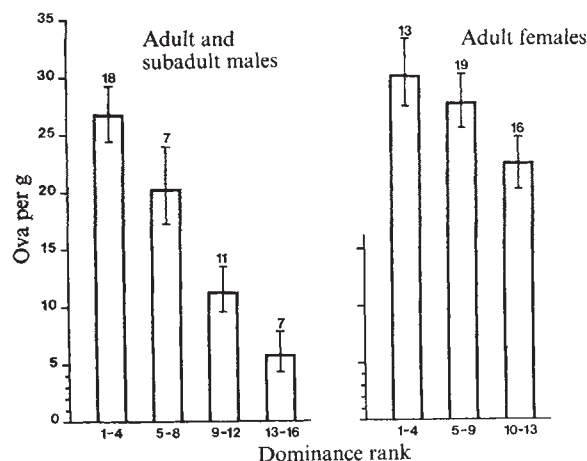


Fig. 1 Parasite egg counts in relation to dominance rank. Shown are mean egg counts for each dominance rank class and 95% asymmetrical confidence intervals for the mean. Figure at top of each bar gives number of samples on which mean is based. For adult males, analysis included only samples collected during periods of rank stability. Subadult males are the same age as young adult females.

adult female baboons rarely change dominance rank, but changes in their reproductive state or menstrual cycle phase occur frequently⁴. Sexually cycling females had significantly higher egg emissions than did anoestrous (lactating or otherwise non-cycling) females ($F_{max} = 3.26$, d.f. = 2,250, 206, $P < 0.01$); anoestrous females in turn had significantly higher egg emissions than did pregnant females ($F_{max} = 2.14$, d.f. = 206, 96, $P < 0.01$) (Fig. 2). Among sexually cycling females, mean egg counts were significantly higher during the one week immediately before and the one week immediately after the onset of menstruation compared with the remaining 3 weeks mid-cycle that include oestrus and ovulation ($F_{max} = 5.65$, d.f. = 1,912, 338, $P < 0.01$) (Fig. 2).

The mean egg count for adult females, 26.58 ova per g, was significantly higher than the mean egg count for adult males during periods of rank stability, 20.69 ova per g ($F_{max} = 1.28$, d.f. = 2,552, 1,490, $P < 0.01$), and this difference was even greater when subadult males and adult males inconsistent in their dominance relationships were included in the comparison. Parasite ova emissions among adult females, however, were not correlated with dominance rank ($r_s = 0.02$, $P > 0.05$), although middle ranking females did have significantly higher egg counts than did low ranking females ($F_{max} = 1.24$, d.f. = 1,054, 714, $P < 0.01$) (Fig. 1).

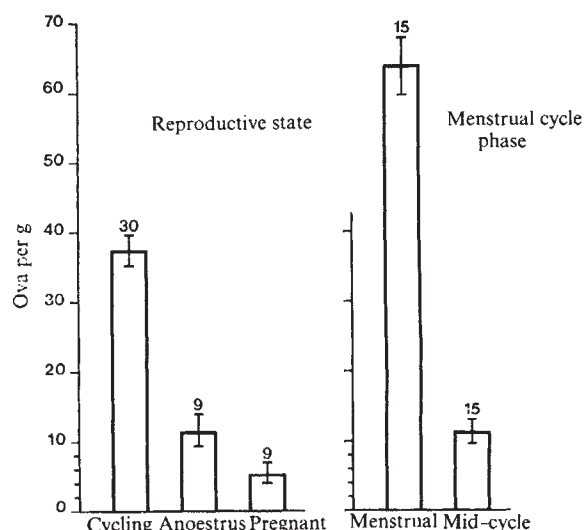


Fig. 2 Parasite egg emissions in relation to female reproductive state and menstrual cycle phase. Conventions as in Fig. 1.

One simple but plausible explanation for the observed pattern of parasite ova emissions is that individuals differ in ingestion rate of infective and non-infective ova or in other aspects of food intake, gut passage time or faecal output. Alternatively, high ranking adult males and sexually cycling females may provide a generally more favourable internal environment for egg production by parasites than do individuals of other social and reproductive classes. Of course, the above correlations might also reflect actual differences in the burden of adult worms harboured by individuals of each social and reproductive class. Although unlikely, it is also possible that parasite ova emissions affect social and reproductive condition, for example, dominance rank, rather than the other way around as presumed by the above alternatives. Regardless of their precise

explanation, the relationships described in this note, if substantiated by more extensive studies, will be of interest and importance to many scientists concerned with primates, parasites or public health.

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Inhibition of *Leishmania donovani* transformation by hamster spleen homogenates and active human lymphocytes

HAEMOFLAGELLATES are protozoan parasites responsible for many important diseases of man and domestic animals¹. *Leishmania donovani*, the cause of visceral leishmaniasis or kala azar, is a widespread human parasitic haemoflagellate. Its life cycle involves man (and other mammals) and an insect vector (sandflies) with a different stage in each of the two host types. Transformation from one stage to the next includes morphological antigenic, physiological, and infectivity changes²⁻³.

Transformation in these organisms is a fundamental developmental transition whose regulation is incompletely understood. In this report we present evidence that transformation from the mammalian (amastigote) stage of *L. donovani* to the insect and/or culture (promastigote) stage is inhibited by host hamster spleen homogenates and by a soluble factor(s) synthesised by activated human lymphocytes. In the absence of this factor transformation proceeds in an orderly series of steps (Fig. 1).

Synchrony of transformation by a population of amastigotes is rather poor; transformation to the promastigotes begins after 18 h with all cells capable of developmental transition (~60-80% of the cells undergo transformation) completing cytodifferentiation by about 60 h (Fig. 2). Transforming amastigotes do not incorporate ¹⁴C-thymidine⁴

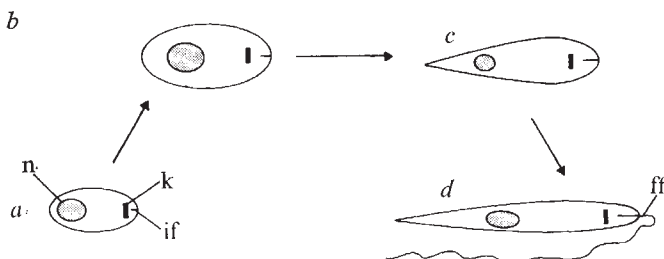


Fig. 1 Morphological events during transformation of a *L. donovani* amastigote to the promastigote stage. *a*, Oval-shaped amastigote with nucleus (*n*), kinetoplast (*k*) and internal flagellum (*if*), 0-8 h; *b*, tenfold enlargement of the oval stage, ~8-16 h; *c*, elongation of the enlarged parasite, ~16-18 h; *d*, formation of free flagellum (*ff*) and further elongation to complete cytodifferentiation, ~18-20 h. Amastigotes were obtained as

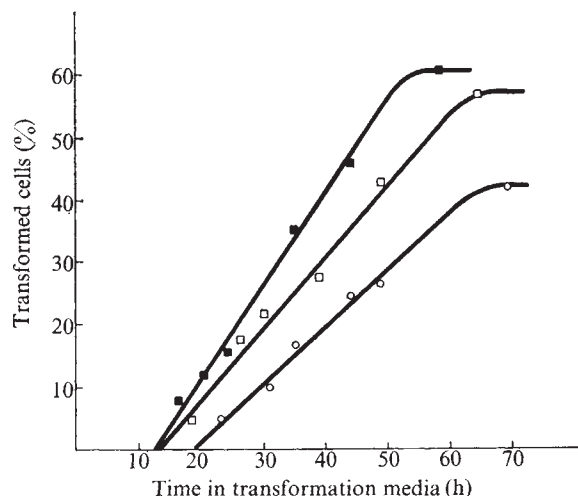


Fig. 2 Transformation of *L. donovani* amastigotes to promastigotes: influence of preincubation in glucose-free Locke's solution on the times of initiation and completion of transformation by a population of washed amastigotes. ○, Control amastigotes transferred to transforming medium (incubated at 27 °C) immediately after isolation from hamster spleen; □, amastigotes preincubated for 8 h at 27 °C before transfer to transformation medium; ■, amastigotes preincubated for 8 h at 34 °C before transfer to incubation medium. Amastigotes (~10⁸ cells) were incubated in 25-ml Erlenmeyer flasks containing 5 ml HO-MEM (containing glucose). Samples were removed from flasks at various times and the percentage of transformed cells (stage *d* in Fig. 1) determined by microscopic observation. In all experiments the final samples were tested for bacterial contamination; the data from contaminated flasks were discarded.

into DNA; however, promastigotes readily incorporate this substrate into DNA (~70% of recoverable ¹⁴C from labelled thymidine is found in DNA). This suggested that *de novo* DNA synthesis does not occur in transforming amastigotes.

We then incubated freshly isolated amastigotes in glucose-free Locke's solution (pH 7.7) for 8 h at 27 °C before transfer to transformation medium (HO-MEM)⁷ to deplete possible contaminant compounds carried over during isolation that might inhibit cytodifferentiation. The cells transformed more synchronously and the times required for initiation and completion of transformation were markedly reduced (Fig. 2). Even shorter initiation and completion times for developmental transition of amastigote populations were found when cells were preincubated in glucose-free Locke's solution for 8 h at 34 °C before transfer to HO-MEM (Fig. 2).

To determine if a host factor(s) carried as a contaminant with amastigotes during their isolation delayed transformation, 2.5 ml of the first wash from infected spleen homogenates (see legend to Fig. 1) was added to 2.5 ml of

follows: male hamsters were killed with ether about 2 months after intraperitoneal inoculation with freshly isolated amastigotes (Malakal area Sudan strain, 1S) and the spleen excised in sterile conditions. The spleen was homogenised in 10 ml Tris (0.2 M)-sucrose (0.25 M) buffer, pH 7.8, with a Ten Broeck glass homogeniser. Homogenates were centrifuged at 150g for 10 min and the pellet discarded; the supernatant was centrifuged at 1,000g for 20 min and the resultant supernatant (first wash) discarded. The pellet was resuspended in 10 ml Tris-sucrose buffer and this procedure repeated two more times. After resuspending the final pellet (washed amastigotes) in a small amount of Tris-sucrose buffer, amastigotes were counted and 10⁸ cell aliquots incubated in HO-MEM⁷ medium at 27 °C. Isolation procedures were carried out in the cold to retard spontaneous transformation, and sterile techniques were used to prevent bacterial contamination. The cell depicted was preincubated in glucose-free Locke's solution (see Fig. 2). About 10% of the preincubated cells started to transform after 8 h in transforming medium. Although stage *a* lasts longer for non-preincubated cells, the duration of time spent in the other stages is the same as that for preincubated cells.

Table 1 Secretion of factors inhibiting *L. donovani* amastigote to promastigote transformation by cell culture systems*

Cell line tested	Effect of cell culture† on transformation	Effect of conditioned‡ medium on transformation
<i>Triatoma infestans</i> embryo cells BTC-32	—†	
Mouse sarcoma 338	—	
Mouse lymphocyte sarcoma 815	Inhibited 3–5 d (8)§	Delayed 12–20 h (8)
Mouse fibroblast L cells	—	
Mouse glial tumour C6	—	
Mouse transformed fibroblast THC-1	—	
Bovine embryonic trachea EBTR	—	
Dog kidney (epithelium) with mycoplasma mdck	Inhibited 5 d (3)¶	Delayed 24–48 h (3)
Monkey fibroblasts TC7	—	
HeLa	—	
Primary human lymphocytes (from adenoid and tonsil)	Inhibited 3–5 d (5)¶	Delayed 12–20 h (5)
Phytohaemagglutinin-activated primary human lymphocytes	Inhibited at least 5 d (5)¶	Delayed 24–48 h (5)

*10⁸ washed amastigotes in each flask, incubation temperature 27 °C, transformation determined as in Fig. 2.

†Control amastigotes incubated in absence of cells in same media used to grow tissue cells: RPMI 1640 (GIBCO) or Hanks' MEM (GIBCO).

‡—, No effect on transformation.

§Culture medium RPMI 1640.

¶Culture medium MEM.

Number of experiments in parentheses.

||Amastigotes inhibited for 3 d or more were washed and transferred to fresh media lacking culture cells to ensure that they were viable and still capable of transformation.

2×HO-MEM before incubation with washed Locke's solution preincubated amastigotes. Addition of the first wash delayed initial transformation times by 12–24 h (four experiments) compared with control cells incubated in 2.5 ml Tris-sucrose buffer and 2.5 ml 2×HO-MEM. Similar results were found when the first wash from uninfected spleen homogenates was tested. Supernatants obtained after the first wash had no effect on amastigote transformation.

Analysis of hamster spleen transformation-blocking activity is difficult because the spleen is small (1–3 g in infected hamsters) and the concentration of the factor(s) responsible for inhibition is low; 1:1 dilution of the first wash before adding it to test media resulted in total loss of activity. We decided to find a model system capable of 'mimicking' hamster spleen transformation-blocking activity and which produced sufficient amounts of factor(s) to analyse inhibition of transformation. Since many developmental processes are mediated by mammalian growth and activating factors (for example, nerve growth factor, epithelial growth factor and lymphocyte-activating factor)^{8,9}, we therefore tested a number of cell culture systems for their ability to secrete high concentrations of factors capable of inhibiting *L. donovani* amastigote cytodifferentiation.

Conditioned media from four mammalian cell culture lines were able to delay initiation of transformation for at least 12 h: mouse lymphocyte sarcoma cell line 815, phytohaemagglutinin-activated primary human lymphocytes¹⁰, unactivated primary human lymphocytes and mdck, a dog kidney cell line infected by an unidentified mycoplasma (Table 1). Activated primary human lymphocytes and mdck both produced high levels of blocking factors. Further experiments were carried out with only the activated lymphocyte system because it was more active and was better defined than mdck in that it was not infected by mycoplasma. Collection of a large volume of media conditioned by the lymphocytes is relatively simple¹⁰; this is another advantage with the lymphocyte system.

Incubation of washed amastigotes in medium conditioned by activated lymphocytes delayed the start of developmental transition by 24–48 h (Table 1). Amastigotes incubated with lymphocyte cultures were inhibited from transforming for at least 5 d. When these amastigotes were washed and transferred to fresh media free of lymphocytes cytodifferentiation proceeded normally.

Conditioned medium from activated lymphocyte cultures

was separated by ultrafiltration into fractions containing molecules of decreasing molecular weight; these fractions were assayed for transformation-inhibiting activity (Table 2). Activity was localised in fraction IIIc (molecular weight <500); addition of fraction IIIc to amastigotes in HO-

Table 2 Transformation-inhibiting activity in water-soluble fractions secreted by phytohaemagglutinin-activated primary human lymphocytes*

Test material† (molecular weight)	Effect on transformation‡
Fraction I (>30,000)	—
Fraction II (5,000–30,000)	—
Fraction III (<5,000)	Delayed 48–72 h (5)§
Fraction IIIa (1,000–5,000)	—
Fraction IIIb (<1,000)	Delayed ≈48 h (3)
Fraction IIIb' (500–1,000)	—
Fraction IIIc (<500)	Delayed ≈48 h (3)

*Details of primary human lymphocyte preparation, cell culture and collection of conditioned media have been published¹⁰. Lymphocytes were cultured in MEM supplemented with essential amino acids, glutamine, sodium pyruvate and with 10% foetal calf serum. Cells were activated by addition of 20 µg ml⁻¹ phytohaemagglutinin (PHA-P, Difco) and incubated for 5–7 d. Cultures were centrifuged at 300g for 5 min to pellet the lymphocytes, and the supernatant saved. Supernatant could be stored frozen or separated into different fractions and then stored frozen.

†Supernatants separated by ultrafiltration into fractions with solutes of different approximate molecular weight. Filtration with an Amicon PM30 resulted in a concentrated fraction of molecular weight >30,000 (fraction I) and a filtrate containing solutes of molecular weight <30,000. The filtrate was further concentrated with an Amicon PM10; the concentrate contained solutes of about 5,000–30,000 molecular weight (fraction II). The material passing through with solutes of molecular weight <5,000 (fraction III) was concentrated using an Amicon UM2; this concentrate is defined as fraction IIIa (solutes of about 1,000–5,000 molecular weight). The material going through the UM2 is defined as fraction IIIb (solutes of molecular weight <1,000). Fraction IIIb was concentrated with an Amicon UM05; the concentrate is defined as fraction IIIb' (solutes of molecular weight 500–1,000) and filtrate is fraction IIIc (<500 molecular weight).

‡Amastigotes (10⁸ cells) incubated in 5.0 ml HO-MEM containing test fraction; concentrated HO-MEM was added to concentrated test fraction in the proper proportions for a final concentration of 1×HO-MEM and 1×test fraction. Transformation was determined as in Fig. 2. Cells were not permanently affected by inhibitory fractions; after delay of transformation the number of amastigotes completing cytodifferentiation was similar to that of controls. Control amastigotes were incubated in 5.0 ml HO-MEM. In all experiments the final samples were tested for bacterial contamination and the data from contaminated flasks discarded.

§Number of experiments in parentheses.

MEM delayed initiation of cytodifferentiation by ~48 h. The solutes in fraction IIIc are present in fractions III (molecular weight <5,000) and IIb (molecular weight <1,000); as would be expected, the latter two fractions show transformation-blocking activity. The lower activity found in fractions IIb and IIIc may be due to inefficient passage of filtrate through the Amicon UM2 filter membrane. This fraction is relatively stable; it is not inactivated by boiling for 30 min and can be stored at 4 °C for 5 d or at -17 °C for at least several months with little loss of activity.

Transforming amastigote populations can be blocked at any time before the process is complete. Addition of fraction IIIc to amastigotes after 9 h incubation in HO-MEM delayed initiation of cytodifferentiation by about 9 h. Addition of the fraction after 27 h incubation, a time when a few of the cells have already transformed, delayed further transformation for about 15 h. These are unusual results because in most developmental systems there is a point when cells are irreversibly committed to change independent of the initial factor(s) causing that change.

Addition of fraction IIIc or of the first wash from hamster spleen homogenates had no effect on promastigote cultures in HO-MEM. The cells maintained their normal morphology and continued to divide.

The above results present the first evidence for the presence of mammalian regulatory compounds affecting haemoflagellate transformation. There are factors produced by activated human lymphocytes and in hamster spleen homogenates that inhibit transformation of *L. donovani* amastigotes to the promastigote stage. The *in vitro* studies by Lamy¹¹ with macrophage and Sticker sarcoma cell cultures demonstrating that *L. donovani* amastigotes may occasionally transform into promastigotes while still inside the host cells, can be explained by the possible absence of 'blocking' factors in his cultures.

It remains to be confirmed whether one or more inhibitory factors is present in the lymphocyte secretions and to determine if blocking factors from hamster spleen and cell lines 815 and mdck are similar to those produced by lymphocytes.

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Generation of cytotoxic lymphocytes *in vitro* against autologous human leukaemia cells

NORMAL lymphocytes cultured with allogeneic cells in one-way mixed leukocyte culture (MLC) respond proliferatively to the 'lymphocyte-defined' (LD) antigens and subsequently cytotoxic cells are generated against the target antigens on the stimulating cells¹. In contrast, when responding lymphocytes are cultured with stimulating lymphocytes that differ with respect to target antigens but not LD antigens, proliferative and cytotoxic responses are not easily generated; the addition of a second stimulating cell differing from the responding cell with respect to LD antigens in a 'three-cell' experiment allows the generation of cytotoxic lymphocytes directed against target antigens¹⁻⁴. Consistent with results of others⁵⁻⁹, we have detected low but significant MLC responses after culturing remission lymphocytes with irradiated autologous leukaemia cells; however, cytotoxic lymphocytes were not generated. Using the 'three-cell' approach, where remission lymphocytes, irradiated leukaemia cells and irradiated allogeneic stimulating cells were cocultured, we have generated lymphocytes specifically cytotoxic for autologous human leukaemia cells.

Peripheral blood was obtained at the time of diagnosis from C.T., a 40-yr-old woman with acute myelomonocytic leukaemia (AMML). The peripheral white blood cell count at this time was 200,000 per mm³, consisting of 52% blast cells. The blood was diluted with medium 199 and layered on a Ficoll-Hypaque gradient¹⁰; the resulting cells at the interface consisted of 74% blast cells. These cells were washed and control-frozen in RPMI 1640 with 25 mM HEPES buffer and 20% heat-inactivated normal human serum and stored in liquid nitrogen. Eight and twelve weeks after diagnosis, after several courses of intensive chemotherapy, peripheral blood and bone marrow aspirates were obtained. At these times, no leukaemic cells were present in the peripheral blood. Lymphocytes were separated from peripheral blood by Ficoll-Hypaque sedimentation and suspended in culture medium consisting of RPMI 1640 with 25 mM HEPES buffer and 20% heat-inactivated normal human serum. Specimens of bone marrow aspirate obtained at these times were purified in the same way. Approximately 5% of the marrow cells collected from the interface of the gradient were blast cells.

Cultures were set up in 50-ml Falcon flasks, each containing 5×10^6 responding lymphocytes (either the patient's remission lymphocytes or lymphocytes from normal individuals) and $5-10 \times 10^6$ stimulating cells rendered incapable of DNA synthesis by exposure to 2,500 R X irradiation. Stimulating cells were either irradiated leukaemia cells, allogeneic lymphocytes, a mixture of leukaemia cells and allogeneic lymphocytes or a mixture of bone marrow cells and allogeneic lymphocytes. Cultures were incubated at 30 °C in a humidified 5% CO₂ incubator and were fed with 5 ml of culture medium on days 2 and 5. Target cells, to be used on day 7 in the cell-mediated ⁵¹Cr release assay (CML) were prepared by culturing either $5-15 \times 10^6$ normal lymphocytes or bone marrow cells in flasks that were fed on days 2 and 5. The leukaemic cells were thawed on the day of the CML assay, incubated for 2 h at 37 °C before labelling with 250 µCi ⁵¹Cr (Na₂ ⁵¹CrO₄, New England Nuclear). Effector cells generated in flasks were collected on day 7 and the CML assay was performed as described in the legend of Table 1a and b.

In our first experiment shown in Table 1a, C.T. remission lymphocytes cultured with autologous leukaemia (L_x) cells alone were not cytotoxic for that patient's leukaemia cells—less ⁵¹Cr was released in the presence of CL_x effector cells than was released spontaneously. In contrast, lymphocytes that had been cultured with a mixture of autologous leukaemia cells (L_x) and allogeneic lymphocytes (A_x) were cytotoxic for the patient's

Table 1 *In vitro* generation of lymphocytes specifically cytotoxic for autologous AMML cells

Responding lymphocytes		Stimulating cells	Effector-target-cell ratio	Leukaemic cells	% Specific Remission marrow cells	^{51}Cr release $\pm$ s.d. Normal lymphocytes from A	Normal lymphocytes from B
(a)							
C	(patient's lymphocytes)	L_x (patient's leukaemia cells) + A_x (normal allogeneic lymphocytes)	40:1	17.8 $\pm$ 6.5	3.5 $\pm$ 1.4	44.0 $\pm$ 4.1	1.8 $\pm$ 2.3
C	(normal individual)	L _x	40:1	0.0	0.0		
C		A _x	40:1	2.9 $\pm$ 1.1	0.45 $\pm$ 1.6	60.7 $\pm$ 10.1	2.2 $\pm$ 2.2
B		L _x	20:1	17.5 $\pm$ 12.0	14.7 $\pm$ 2.0	-0.7 $\pm$ 3.4	-0.4 $\pm$ 2.8
B		A _x	20:1			27.7 $\pm$ 2.3	-0.2 $\pm$ 3.4
(b)							
C	(patient's lymphocytes)	L_x (patient's leukaemia cells) + D_x (normal allogeneic lymphocytes)	20:1	25.0 $\pm$ 6.9	Normal lymphocytes from D 30.3 $\pm$ 1.7	Normal lymphocytes from E 7.2 $\pm$ 3.6	
C		M _x (remission marrow)					
		+ D _x	20:1	22.0 $\pm$ 8.9	39.7 $\pm$ 3.5		
C		C _x (patient's remission lymphocytes)	20:1	0.0	0.0	0.0	
D		(normal individual)	L _x	20:1	36.8 $\pm$ 6.5	-2.4 $\pm$ 4.1	

In both experiments *a* and *b*, lymphocytes were collected 7 d after stimulation, resuspended at 1×10^6 – 2×10^6 cells ml^{-1} and 0.2 ml effector cells were mixed with 1×10^4 ^{51}Cr -labelled target cells in wells of round-bottom Linbro plates. Supernatants were collected after 6 h (Exp. *a*) or 8 h (Exp. *b*) incubation at 37 °C and % specific ^{51}Cr release was calculated according to the following formula: $[(E - SR)/(M - SR)] \times 100$, where *E* is experimental release, *SR* is spontaneous release of ^{51}Cr from normal target cells in the presence of medium alone, and *M* is maximal release of ^{51}Cr from target cells after adding detergent (0.4% Cetrimide). Specific ^{51}Cr release from leukaemic cells and bone marrow cells was calculated from the following formula: $[(E - C)/(M - C)] \times 100$, where *E* is experimental release, *C* is release of ^{51}Cr from target cells by CL_x or CC_x effector cells and *M* is maximal release.

leukaemia cells: 17.8% specific ^{51}Cr released observed by 6 h. These same effector cells that were cytotoxic for autologous leukaemia cells and A target cells did not lyse unrelated normal lymphocytes from B and caused only 3.5% ^{51}Cr release from autologous bone marrow cells containing 5% blast cells. It should be noted, however, that these remission bone marrow cells and leukaemia cells were nearly equally sensitive to lysis by allogeneic normal lymphocytes (B) that had been cultured with the patient's leukaemic (L_x) cells. Remission lymphocytes that had been cultured with allogeneic (A_x) cells were highly cytotoxic for A target cells (60.7% specific ^{51}Cr release) but caused only 2.9% ^{51}Cr release from autologous labelled leukaemic cells. This indicates that lysis of autologous leukaemia cells by remission lymphocytes that had been cultured with a mixture of A_xL_x stimulating cells was not due to cross reacting target antigens expressed on leukaemia cells and allogeneic (A) lymphocytes. Furthermore, the presence of leukaemic stimulating cells in the mixed culture was necessary for the generation of an anti-leukaemic cytotoxic response.

Remission lymphocytes were obtained 1 month later and it was again possible to generate cytotoxic lymphocytes directed against autologous leukaemia cells (Table 1*b*). Remission lymphocytes cultured with leukaemic cells and allogeneic (D_x) cells caused 25% ^{51}Cr release from leukaemic cells after an 8-h CML assay at the ratio of 20 effector cells per target cell and 30% ^{51}Cr release from allogeneic D target cells. Nearly the same amount of ^{51}Cr (22%) was released from leukaemic cells by lymphocytes that were cultured with bone marrow and allogeneic cells. A probable explanation for this finding is that the number of blast cells carrying target antigens in the stimulating marrow population was sufficient to induce a cytotoxic lymphocyte response. In the experiment shown in Table 1*a*, however, lymphocytes that had been cultured with leukaemic and allogeneic cells caused only 3.5% ^{51}Cr release from labelled marrow cells, for only a

minor proportion (5%) of the ^{51}Cr -labelled target cells were blasts, many of which may not have been leukaemic.

The results presented here demonstrate the presence of target antigens on AMML cells against which a cytotoxic autologous lymphocyte response was generated when a strong proliferation-inducing signal (in this case, allogeneic stimulating cells) was provided that presumably stimulates the proliferating helper T cells¹. The relationship between the target antigens we have detected on AMML cells by cell-mediated cytotoxicity and antigens detected serologically on AMML cells¹¹ is unknown. We have not had access to cells of other patients with AMML; however, consistent with the results we have reported here, we found that in a patient with acute lymphocytic leukaemia, the development of cytotoxic response aimed at the patient's leukaemia cells was again dependent on the providing of a helper stimulus in a three-cell protocol. Similar experiments with five patients in remission from acute myelogenous leukaemia (AML) have not as yet resulted in generation of cytotoxic lymphocytes directed against autologous leukaemic cells. In some of these cases, the AML cells did not generate cytotoxic activity even in allogeneic responder lymphocytes. The explanation for the lack of finding of cytotoxicity in cases where allogeneic lymphocytes did respond cytotoxicity to target antigens on leukaemic cells is not clear.

Consistent with our studies are the independently derived findings of Sondel *et al.* (personal communication) showing that adding a helper stimulus enhances anti-leukaemia cell-mediated cytotoxicity by lymphocytes from siblings HLA identical with the leukaemia patient. The 'three-cell' type approach used in these experiments may thus provide a means for generating cytotoxic lymphocytes directed against other autologous or HLA identical leukaemia or tumour cells that express target antigens but cannot induce a strong lymphocyte proliferative response.

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Sensitivity of H-2-less target cells and role of H-2 in T-cell-mediated cytotoxicity

THERE is no definitive teleological interpretation of the involvement of H-2 determinants in the recognition by mouse cytolytic T cells of various non-H-2 antigens^{1,2}. While it is generally believed that T-cell immunity against non-H-2 structures (determined, for example, by infectious agents) may benefit from the known special reactivity of T cells against non-self H-2, other interpretations have been proposed, such as the possibility that H-2 is necessary for cytotoxicity at a postrecognition stage as the "weak point" of the target-cell surface³. A prediction of this hypothesis is that target cells devoid of H-2 should be resistant to T-cell-mediated cytotoxicity. We have tested this prediction using male germinal cells, embryonal carcinoma and virus-transformed testicular cells as targets, in the presence of the lectin concanavalin A (con A). Con A binding apparently by-passes⁴ the normal recognition system, so that lack of recognition of H-2 on H-2-less target cells would not be a limiting factor for cytotoxicity. We found that H-2-less embryonal carcinoma cell lines, including one with barely detectable levels of F9 antigen, were sensitive to lysis, strongly suggesting that at a postrecognition stage H-2 is not required for T-cell-mediated cytotoxicity.

Mouse male germinal cells, in practice testicular cells, acquire serologically detectable surface H-2 as late as 12 d of age^{5,6}. We wondered whether acquisition of H-2 would be accompanied by a change from resistance to sensitivity to lysis. We found, however, that even testicular cells from several-month-old mice were relatively resistant to lysis, in the presence or absence of con A (Table 1). Further findings (not shown) were: first, ⁵¹Cr could be released from testicular cells in the presence of rabbit anti-mouse serum and complement; second, con A induced agglutination of testicular cells at the concentration used in these experiments; third, various ratios of cytolytic to testicular cells and various concentrations of con A did not yield different results. These experiments showed at least that the presence of serologically detectable H-2 was not sufficient to ensure sensitivity to lysis, thus underlining the existence of other, unknown target cell requirements for lysis.

To investigate whether H-2 was necessary for lysis, we then used as targets F9 embryonal carcinoma cells of H-2b genotype but devoid of serologically detectable membrane H-2 (ref. 7). Table 2, experiments 1–3, shows, first, that F9 cells were not lysed by anti-b cytolytic cells in the absence of con A, and second, that a significant degree of lysis occurred in the presence

Table 1 Lack of demonstrable T-cell-mediated cytotoxicity of mouse testicular cells

Experiment	Target cells*	Cytolytic cells			
		Con A†		k anti-d	
		–	+	–	+
(1)	P815	51	49	2	29
	Test. CBA 3w	–1	1	–3	1
(2)	P815	32	31	0	16
	Test. CBA 3w	0	0	1	–2
	Test. CBA 5w	–1	0	–2	1
(3)	P815	37	41	1	42
	Test. CBA 5w	–5	2	1	4
(4)	Blasts BALB/c	28	28	1	22
	Blasts C3H	1	16	29	31
	Test. BALB/c 6w	7	6	–1	5
	Test. C3H 6w	0	2	1	4
(5)	Blasts BALB/c	24	5	–3	18
	Blasts C3H	–2	9	30	20
	Test. BALB/c 4m	–5	–4	–3	4
	Test. CBA 7m	–1	–3	–2	6

C3H anti-BALB/c (k anti-d) and BALB/c anti-C3H (d anti-k) sensitised cytolytic cells were prepared and tested in a ⁵¹Cr-release assay as described previously¹⁸, at an effector–target cell ratio of 20:1 in these experiments. Cytotoxicity is expressed as percentage ⁵¹Cr release averaged from triplicate wells, minus spontaneous release by target cells alone. In this type of assay, an experimental difference of 5% or more is statistically significant ($P = 0.05$).

*Target cells (prelabelled with ⁵¹Cr, 10⁴ per V-shaped well of microplates) were P815 mastocytoma cells (H-2d), either CBA and C3H (H-2k) or BALB/c (H-2d) mechanically dispersed testicular cells from mice aged 3 weeks to 7 months, and con A-induced blasts (obtained through 2–4-d incubation of normal spleen cells in the presence of con A (2.5 μg ml^{–1}) then labelled with ⁵¹Cr and incubated for two consecutive periods of 15 min with 0.1 M α-methylmannose). Spontaneous release was 9–15 for P815, 20–41 for testicular cells and 14–35 for con A blasts.

†Con A at a final concentration of 10 μg ml^{–1} consistently induced in the presence of sensitised cells nonspecific cytotoxicity of most target cells tested except testicular cells. It sometimes induced a decrease of specific cytotoxicity, especially marked here in experiment 5.

of con A. This dual pattern was observed in each of eight experiments, not only with d anti-b and b anti-d, but also with k anti-b and b anti-k cytolytic cells (not shown). Lysis in the presence of con A showed that F9 cells are sensitive to lysis (which incidentally strengthens the conclusions drawn from interesting negative results obtained with TNP-coated F9 cells⁸). That no lysis should occur in the absence of con A therefore strongly suggests that specific anti-H-2 cytolytic cells do not recognise H-2 determinants at the surface of F9 cells. Thus, F9 cells are sensitive to lysis in the presence of con A, in spite of the absence of any H-2 determinant detectable either by specific antisera⁷ or by specific cytolytic cells.

F9 cells, however, bear the F9 embryonic antigen⁹ and in view of the claimed homology¹⁰ between H-2 and F9 one can argue that the F9 antigen can serve as a substitute for H-2 in lysis. This led us to use strain PCC3/A/1/D-G1, derived from the primitive embryonal carcinoma cell line PCC3/A/1 (ref. 11). PCC3/A/1/D-G1 cells bear neither H-2 nor F9 antigens detectable with antisera and complement and have at least 100 times less F9 antigen than F9 cells in adsorption experiments and only about 1–2% positive cells in an anti-F9 antigen immunofluorescence assay¹¹. Table 2, experiments 4–6, and Table 3 show that cytotoxicity of PCC3/A/1/D-G1 cells followed a pattern similar to that of F9 cells.

Con A-dependent lysis of H-2-less target cells was T-cell-mediated, since it was significantly decreased by anti-Thy 1 serum in the presence of complement, in a specific brain-adsorbable manner (Table 3). Using H-2-bearing target cells, it was found, first, that con A-dependent nonspecific lysis is probably mediated by T cells also capable of mediating specific lysis^{4,12–14}, and second, that the same drugs inhibit with similar dose–response curves the lethal hit stage of specific cytotoxicity¹⁵ and con A-dependent nonspecific lysis (P.G., unpublished results). It therefore seems reasonable to assume that both types of T-cell-mediated lysis differ only at the recognition stage and

Table 2 Sensitivity to T-cell-mediated cytotoxicity of embryonal carcinoma and virus-transformed testicular cell lines

Experiment	Target cells*	Serotype†		Cytolytic Cells‡							
		H-2	F9	Con A: — 20:1§	b anti-d		+ 5:1		d anti-b		+ 5:1
1	EL ₄	b	—	3	2	29	24	29	14	27	21
	F9	—	+	0	1	13	3	—1	—1	10	12
2	EL ₄	b	—	8	2	23	18	38	19	38	25
	P815	d	—	40	14	44	17	2	1	39	16
3	F9	—	+	0	2	13	14	0	0	25	17
	TSV	b	+	3	0	4	4	11	6	20	12
4	EL ₄	b	—	—1	0	17	12	24	13	26	16
	PCC3/A/1/D-G1	—	—	—3	—1	14	7	2	0	16	13
5	EL ₄	b	—	0	—2	30	36	44	25	46	35
	P815	d	—	46	32	58	42	8	2	44	34
6	PCC3/A/1/D-G1	—	—	—2	—3	27	20	3	1	26	20
	TSV	b	+	1	0	16	12	13	5	17	11
6	EL ₄	b	—	—1	—3	38	28	38	22	41	27
	P815	d	—	38	17	44	23	2	1	37	24
6	PCC3/A/1/D-G1	—	—	—2	0	18	11	2	0	19	11
	TSV	b	+	0	—1	11	4	9	5	12	4

*Target cells were: EL₄ (C57BI/6 lymphoma) and P815-X2 (DBA/2 mastocytoma) cultivated *in vitro* in standard conditions; F9 (strain 129 embryonal carcinoma) grown in 15% foetal calf serum in gelatinised flasks, used for experiments within 2 months of subculture; PCC3/A/1/D-G1 (derived *in vitro* from mouse strain 129 embryonal carcinoma cell line PCC3/A/1) grown in 15% foetal calf serum, also used within 2 months of subculture; TSV (SV40-transformed mouse strain 129 testicular cells). Spontaneous release was 11–20, 8–10, 15–31 and 13–30, respectively. The embryonal carcinoma cells were especially sensitive to careless handling.

†Serotypes after refs 7, 9, 11 and unpublished results.

‡Cytolytic cells were C57BI/6 anti-BALB/c (b anti-d) and BALB/c anti-C57BI/6 (d anti-b). The latter were usually superior to the former in terms of number of cells recovered after sensitisation, percentage of blasts and efficiency of lysis of all types of target cells in the presence of con A.

§Ratio of cytolytic to target cells.

follow the same mechanism after recognition. If this assumption is not correct, then our conclusions below about the sensitivity of H-2-less target cells would still be valid for some cytolytic T cells, but not necessarily for the specifically cytolytic ones.

In this series of experiments, we also tested as targets TSV cells derived from SV40-transformed mouse testicular cells and expressing both H-2b and F9 antigen (F.K., unpublished). These cells were lysed to a moderate but significant extent by specific anti-b cells with and without con A and by anti-d cells in the presence of con A (Table 2). The availability as T-cell-sensitive targets of H-2⁺ F9⁺ virus-transformed and of H-2⁺ F9⁺ carcinoma cells seems of great potential interest. A study of the induction and expression of anti-F9 antigen autoimmunity as a function of the presence of H-2 may help eventually to reveal not only a genetic¹⁶ and ontogenic¹⁰, but also an immunological relationship between H-2 and F9. It is clear from the results reported here that the presence of F9 determinants does not prevent recognition by T cells of H-2 determinants at the target cell surface.

The nature of the primary target cell lesion caused by cytolytic T cells is unknown. Molecular channels can be inserted into the target cell membrane, as in the "doughnut" model proposed by Mayer for complement-mediated lysis¹⁷. It may also be that

'target molecules' on the target cell membrane have a role, for example as substrates for effector cell membrane enzymes. If this is so, this report shows that neither H-2 nor very probably F9 are the exclusive target molecules, or are exclusively associated with target molecules, since lysis can proceed in their apparent absence. This strongly suggests there is no strict requirement for H-2 at a postrecognition stage, which would account for the H-2 restriction of T-cell-mediated cytotoxicity.

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Table 3 Effect of anti-Thy 1 serum on cells mediating con A-dependent lysis of embryonal carcinoma cells

Target cells*	Con A	Cytolytic cells† Anti-Thy 1-2‡	k (Thy 1-1) anti-b none absorbed on Thy 1-1 brain			k (Thy 1-2) anti-b none absorbed on Thy 1-1 brain			absorbed on Thy 1-2 brain	
			—	—	+	—	—	+	—	+
EL ₄	—	Complement	30	28	31	26	21	—2	21	22
	+		34	33	33	27	25	—2	25	24
PCC3/A/1/D-G1	—	Complement	4	—3	2	1	—1	2	—4	0
	+		25	32	25	21	27	6	19	20

*Target cells were EL₄ lymphoma and PCC3/A/1/D-G1 embryonal carcinoma cells. Spontaneous release was 13 and 30, respectively. Cytotoxicity was tested in the presence or absence of con A, at a ratio of 20 effector cells (adjusted before treatment with antiserum) to one target cell.

†Cytolytic cells were either AKR (k, Thy 1-1) or C3H (k, Thy 1-2) sensitised against C57BI/6 cells (b).

‡Thy 1-1 anti-Thy 1-2 (AKR anti-CBA) antiserum was absorbed either on Thy 1-1 or on Thy 1-2 brain cells. Samples of 0.2 ml were then added to 0.1 ml of medium containing 2×10^6 cytolytic cells, for 30 min at 4 °C. After two washes, the cells were resuspended in 0.5 ml of a 1:10 dilution of complement (guinea pig serum adsorbed on mouse spleen cells) and incubated for 30 min at 37 °C. The cells were again washed twice, resuspended in medium and tested for residual cytolytic activity.

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Immune destruction of virus-infected cells early in the infectious cycle

TARGET cells acutely infected with herpes simplex virus (HSV) can be destroyed by human blood mononuclear cells (MC) in the presence of IgG antibody to HSV¹⁻³, a process termed antibody-dependent cellular cytotoxicity (ADCC). The major effector cell which mediates this reaction is a K cell; it possesses surface Fc receptors which permit attachment to IgG antibody-sensitised target cells⁴⁻⁶, although the way in which the lethal hit is delivered is poorly understood. For an immune cytolytic mechanism to be maximally effective in limiting the spread of virus in a host, it should be able to destroy the infected cell at an early stage in the viral maturation cycle, before infectious viral progeny can be produced. This requirement seems to be of particular importance for viruses such as HSV, which can avoid exposure to the neutralising antibody in extracellular fluid by spreading from cell to cell across intercellular bridges^{7,8}. In this report, we demonstrate that the ADCC mechanism can recognise viral surface antigens as early as 2 h after cells are infected with HSV and can produce target cell death by 3 h after infection (PI), well in advance of the first appearance of both viral progeny and the susceptibility of infected cells to lysis by antibody and complement (AbC).

MC and HSV antibody added to HSV-infected target cells 2 h after infection uniformly led to substantial cytotoxicity but did not injure uninfected target cells (Table 1). Target cell damage induced by ADCC was somewhat decreased at each successive sampling period. This diminished cytotoxicity might be explained by the fact that the MC, prepared at the beginning

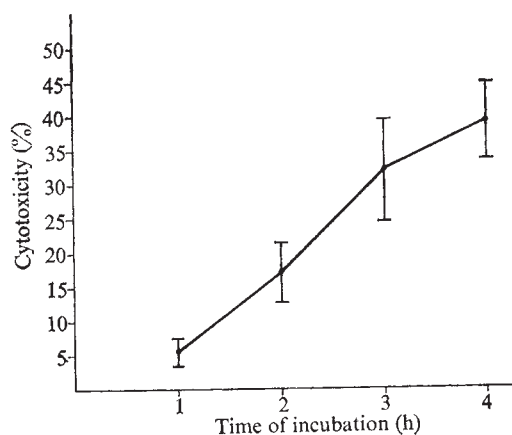


Fig. 1 Kinetics of killing of HSV-infected target cells 2 h PI by antibody-dependent cellular cytotoxicity. The curve represents the mean of four experiments. The vertical bars indicate 1 s.e.m.

of each experiment, may have lost some of their cytotoxic activity while standing at room temperature before use. In contrast to ADCC, AbC did not cause significant target cell damage until at least 8 h after infection, and cytotoxicity equivalent in magnitude to that of ADCC was not seen until 24 h PI. In separate experiments, we found that the amount of ADCC-mediated damage of cells 2 h after infection was related directly to the input of infectious virus. In a representative experiment, cytotoxicity was 43.9% when a relatively high multiplicity of infection (MOI) (15 PFU per cell) was used, 15.7% at a moderate MOI (1 PFU per cell), and only 2.3% at a low MOI (0.1 PFU per cell).

Because the ADCC assay uses an incubation period of 4 h, during which the density of viral surface antigen would be expected to increase, the cells used as targets 2 h after infection may have been lysed at any time between 2 and 6 h after infection. To determine when lysis actually began, we measured the release of ⁵¹Cr from target cells 2 h after infection at hourly intervals during the standard 4-h incubation period. The results (Fig. 1) indicate that a small but significant cytotoxic effect occurred as early as 1 h after the start of incubation, although most of the cell damage occurred during the ensuing 3 h. These findings indicate that some target cells are lysed as early as 3 h after infection starts.

To determine how early after infection HSV-infected cells could be recognised as foreign by the ADCC process, we

Table 1 Lysis of HSV-infected cells at various times by antibody-dependent cellular cytotoxicity and by antibody and complement

Time after infection (h)	Cytotoxicity (%)									
	Expt 1		Expt 2		Expt 3		Expt 4		Expt 5	
	ADCC	AbC	ADCC	AbC	ADCC	AbC	ADCC	AbC	ADCC	AbC
2	36.4	0.0	28.4	0.0	43.9	0.0	49.5	0.2	56.3	
4	32.7	0.0	23.7	3.5	NT	NT	NT	NT	57.6	
8	27.0	3.9	20.5	6.1	NT	NT	NT	NT	51.6	
24	19.6	18.0	21.0	20.1	NT	NT	NT	NT	30.9	
Uninfected	0.0	0.0	0.0	0.0	NT	NT	NT	NT	0.0	

NT, not tested. ⁵¹Cr-labelled Chang liver cells (CL) in suspensions were exposed to the VR3 strain of type 1 HSV at a multiplicity of infection (MOI) of 15 plaque forming units (PFU) per cell for 1 h at 37 °C. The CL were then washed and adjusted to 6–10 × 10⁵ ml⁻¹ in Eagle's minimum essential medium (MEM) with 2% inactivated foetal calf serum (FCS). The cell suspension was incubated at 37 °C in a humid air–5% CO₂ atmosphere. The time of transfer to the incubator was designated zero hour PI. At various times PI, portions of the cell suspension were frozen at –70 °C for determination of infectious virus titres. The remaining cells were chilled in an ice-water bath, washed by centrifugation at 4 °C, and then adjusted to 5 × 10⁴ ml⁻¹ in MEM with 10% FCS for assessment of their susceptibility to lysis by ADCC and lysis by AbC. ADCC and AbC were assayed as before³, except that the incubation period (4 h) for ADCC was shorter. The effector cells for ADCC were Ficoll-Hypaque separated MC from the venous blood of normal subjects. The effector cell–target cell ratio was 50:1. Fresh guinea pig serum at a final dilution of 1:10 was the source of complement (C) in the AbC assay. The antibody source was a pool of heat-inactivated human serum from ten HSV seropositive donors; it had a lymphocyte-dependent antibody titre of 30,000 and a complement-dependent cytolytic antibody titre of 800 against type 1 HSV-infected CL³. Heat-inactivated pooled serum from six seronegative donors served as a control. The serum pools were used at final dilutions of 1:100 for ADCC and 1:10 for AbC, which have been shown to produce optimal lysis³. Cytotoxicity due to ADCC was defined as the percentage ⁵¹Cr released from target cells in the presence of MC and antibody-positive serum (PS) minus the percentage ⁵¹Cr released from target cells in the presence of MC and antibody-negative serum (NS). PS alone was without effect. Cytotoxicity due to AbC was defined as [(W – X) – (Y – Z)], where the symbols represent the percentage ⁵¹Cr release from cells treated with PS and C (W), with PS and heat-inactivated C (X), with NS and C (Y), and with NS and inactivated C (Z). Background release for infected CL was 4–10% during 4 h except for cells 24 h PI, whose release was as high as 20%. Background release for uninfected cells was 10–15%. All values are the means of triplicate determinations. Significant cytotoxicity was defined as calculated values of at least 5% (ref. 3).

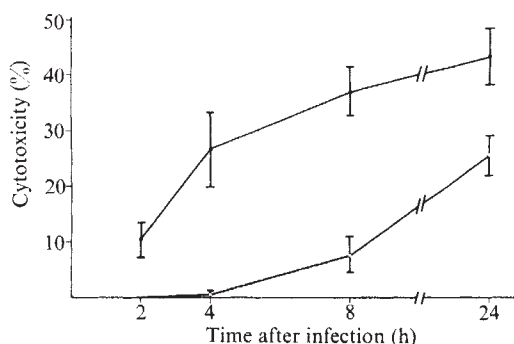


Fig. 2 Recognition of viral antigens on HSV-infected cells at various times after infection by antibody-dependent cellular cytotoxicity (●) and by antibody and complement (○). The curves represent the mean of five experiments in which lysis by ADCC was measured and the mean of three experiments in which lysis by antibody and complement was also measured.

incubated 10^6 CL at various times after infection with 1 ml of a 1:10 dilution of either HSV antibody-positive or HSV antibody-negative serum for 15 min at room temperature. The cells were then chilled in an ice-water bath, and excess unbound antibody was removed by washing them three times at 4 °C with MEM containing 2% foetal calf serum. We confirmed the absence of free lymphocyte-dependent antibody (LDA) by testing the supernates of the final cell washing for LDA activity against HSV-infected CL². Thus, any cytotoxicity shown by these cells after subsequent exposure to MC or complement indicates recognition of viral antigens which were present at the time antibody was added rather than later during the test period. As Fig. 2 shows, ADCC recognised infected cells as antigenically foreign as early as 2 h after infection, whereas AbC, as before, was inactive until 8 h after infection. Pre-sensitisation of target cells followed by removal of free antibody resulted in less ADCC at 2 h after infection than was observed when antibody and MC were added together (as in Table 1). This may be due to two factors. First, when unbound antibody is removed, the free antibody is not available to recognise infected cells that develop a density of viral surface antigen sufficient to support ADCC later than 2 h after infection but before the end of the 4-h test period. Second, the sparseness of viral surface antigens 2 h after infection may not permit firm attachment of antibody to the cell surface so that bound antibody may dissociate during cell washing.

To confirm that the ADCC mechanism recognises and destroys HSV-infected cells before infectious viral progeny

appears, we determined the amount of infectious virus in the CL cultures at various times after infection by plaque titration. The results (Table 2) are typical of four experiments and show that infectious viral progeny were not detected until 8–12 h after infection. On the other hand, viral surface antigen, as measured by direct immunofluorescence, was first noted at 4 h after infection and reached a plateau at 8 h after infection (Table 2). Twenty-five to fifty per cent of the infected, but none of the uninfected cells, also showed a very faint, randomly distributed granular fluorescence within several minutes after exposure to HSV. This fluorescence persisted unchanged until at least 2 h after infection. We interpreted this as indicating the presence on the cell surface of virus which had adhered but not penetrated. Using the binding of radioiodinated antibody to detect viral surface antigen, other investigators have made a similar observation with cells exposed to HSV at a relatively high MOI (ref. 9). Perhaps, by coating the cell surface with antigenic material, this adherent non-penetrating virus may contribute to the early killing of infected cells by ADCC. The quantitative contribution of this phenomenon to ADCC activity is probably small, however, since cytotoxicity seems to be directly related to the *de novo* synthesis of viral antigen by the infected cell (Fig. 2) as indicated by the increase in ADCC with increasing time after infection.

In summary, ADCC can recognise cells infected with type 1 HSV as early as 2 h after infection and produces detectable cell lysis by 3 h after infection. This is well in advance of the appearance of the first mature viral progeny at 8 h PI or later. Consistent with previous studies using HSV-infected cells¹⁰, susceptibility to lysis by antibody and complement is not apparent earlier than 8 h PI. Although we have not directly shown that ADCC inhibits viral replication *in vitro*, our observations suggest that this is a likely consequence of cellular injury at such an early stage in the viral maturation cycle. Because ADCC requires only modest amounts of antibody to operate efficiently¹¹, it should be particularly important in host defence by helping to limit viral spread during the acute phase of a primary infection when specific viral antibody is just beginning to be produced.

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Table 2 Infectious virus titre and membrane fluorescence at various times after infection

Times PI (h)	Virus titre (PFU ml ⁻¹)	Membrane fluorescence (% positive)
0	2.3×10^6	0
2	1.4×10^6	0
4	1.2×10^6	9
6	9.0×10^5	NT
8	1.3×10^6	84
12	6.7×10^6	NT
24	5.0×10^7	95

For determination of total virus at various times PI, cell suspensions (7×10^5 per ml) were frozen and thawed three times, and 0.1-ml samples were plated on to CL monolayers for 1 h at 37 °C. The monolayers were incubated for 5 d at 37 °C in a CO₂ incubator under a semi-solid overlay of 0.7% Agarose before staining with crystal violet and counting of plaques. Surface membrane fluorescence at various times PI was assessed by incubating $3-5 \times 10^5$ cells with 0.1 ml of a 1:15 dilution of a fluorescein-conjugated rabbit anti-HSV serum (supplied by A.J. Nahmias, Emory University) for 15 min at 37 °C. The cells were washed three times in ice-cold normal saline. At least 200 cells per preparation were then examined for surface fluorescence under ortho-illumination in a Zeiss fluorescence microscope. Cells with partial or complete rims of fluorescence were counted as positive.

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Increased ornithine decarboxylase activity in murine sarcoma virus infected cells

POLYAMINE biosynthesis is one of the earliest events occurring during cellular proliferation^{1,2}, and the rate at which it proceeds³ is limited by ornithine decarboxylase (ODC), an enzyme which catalyses the formation of putrescine from ornithine. ODC levels are low, but stable, in resting cells, and a large increase in activity occurs rapidly after the onset of growth^{4,5}. Elevated ODC levels occur in certain tumour cells, including hepatoma, neuroblastoma and SV40-

transformed cells³⁻⁷. Fast growing hepatomas have higher enzyme levels than slow growing ones⁸. During Rous sarcoma virus (RSV) transformation, elevation of ODC levels precedes morphological change⁹.

Here we describe the marked elevation of ODC levels which occurs in murine sarcoma virus (MSV)-infected cells. The continuous, contact inhibited, non-tumorigenic cell line BALB/3T3 (ref. 9) was used for these studies because it has a short doubling time and is readily transformed by MSV. Gz-MSV (ref. 10), which consists of a transforming virus and a non-transforming strain of murine leukaemia virus (MuLV), was grown in BALB/3T3 cells. The MuLV acts as a 'helper' virus, enabling the defective MSV to complete its replication cycle. Cells transformed by MSV in the absence of 'helper' virus contain the sarcoma genome, but do not release infectious virus (non-producer cells)¹¹. Rauscher leukaemia virus¹² (RLV), a non-transforming strain of MuLV, was obtained from the supernatant fluids of chronically infected BALB/c cells. BALB/3T3 cells, clone A31, and KA31, Kirsten MSV virus-transformed non-producer A31 cells, were obtained from Dr Stuart Aaronson.

Table 1 Properties of cell lines

Cell line	Population doubling time (h)	Colony forming efficiency (%)		Virus production (% infectious centres)	
		Liquid medium	Soft Agarose	MSV	MuLV
B/3T3	23	51.5	<0.01	<0.1	<0.1
RLV infected (MOI* 3:1)	23	48.5	<0.01	<0.1	75.0
MSV infected (MOI 1:1)	43	10.5	0.8	46.5	100
KA31	16	48.0	28.0	<0.1	<0.1

Cells were seeded at a density of $5 \times 10^3 \text{ cm}^{-2}$ in growth medium (Dulbecco's modification of Eagle's minimal medium, supplemented with 10% foetal bovine serum and antibiotics). Virus infections were performed in suspension (1 h, 37 °C) before seeding. Total and Trypan blue resistant cell counts were performed 1, 3, 4, 5 and 7 d after seeding. Cells were collected 7 d after seeding and tested for colony forming efficiency in liquid (growth) medium and soft Agarose (0.4%). Virus production was measured 7 d after infection by infections centre assays. Indicator cells were seeded (1×10^6 cells per 60-mm dish) 24 h before addition of test cells, and foci of morphologically altered cells enumerated 5 d later^{12,13}. BALB/3T3 cells were the indicator for MSV production and the S+H- cell line A1-2 for MuLV production.

*MOI, multiplicity of infection.

The properties of the cell lines used are presented in Table 1. RLV infection of BALB/3T3 cells did not alter the growth rate or colony forming efficiency (CFE) in liquid medium. The RLV infected cells remained contact inhibited, and were unable to grow in soft Agarose. KA31 non-producer cells were morphologically transformed, had a short doubling time, and formed colonies when suspended in soft Agarose. MSV infected B/3T3 cells released MSV and MuLV, and were morphologically transformed but their CFE in soft Agarose was low. Their population doubling time was greatly increased and their CFE in liquid medium decreased. Trypan blue uptake studies revealed only a modest increase in the number of non-viable cells (from < 2% in the control cells to up to 8% in the MSV infected cells). The reason for the increased doubling time after acute MSV infection is not understood, but it also occurs in cat cells acutely transformed by the feline leukaemia virus pseudotype of MSV (ref. 14). Apparently many of the cells acutely infected with MSV retain the ability to exclude Trypan blue, but fail to divide, perhaps due to cytogenetic alterations (chromosome breaks and pulverisation) induced by MSV (P. Fischinger, personal communication).

ODC levels of BALB/3T3 cells were growth dependent (Fig. 1). Maximum levels, reached during exponential growth, occurred four days after seeding. The decrease in

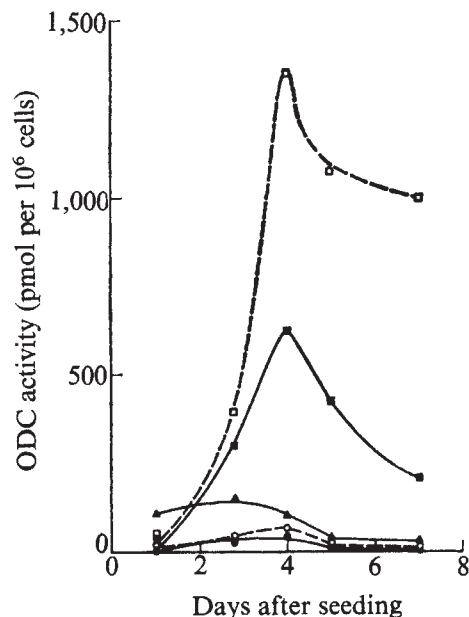


Fig. 1 ODC activities of cell lines. See Table 1. Cell lines were virus infected at the multiplicities (MOI) indicated immediately before seeding. Cells were seeded in 100-mm plastic plates (Falcon) at densities of $5 \times 10^3 \text{ cm}^{-2}$. At 1, 3, 4, 5 and 7 d after seeding, replicate plates of each line were rinsed twice with saline and frozen (-20°C), until assayed. For ODC assays, cells were gently scraped into 1 ml buffer (50 μM EDTA, 25 μM pyridoxal phosphate, 2.5 mM dithiothreitol in 25 mM Tris-HCl, pH 7.1), freeze-thawed three times, and centrifuged (4,500g for 10 min). Supernatant fluids (0.25 ml) were incubated with 50 μl $1\text{-}^{14}\text{C}$ -ornithine (0.2 μCi , 6.25 nmol) in plastic tubes equipped with a rubber stopper supporting a polyethylene centre well. After incubation (37 °C, 45 min), 0.2 ml hydroxide of hyamine was injected into each centre well. After a further incubation of 15 min, 0.2 ml 6% perchloric acid was added to stop the reaction. Tubes were agitated for 15 min to release bound CO_2 , the centre wells were then removed and their radioactivities determined. The experiments were performed five times. Results of a representative experiment are demonstrated. ●, BALB/3T3 control; ○, RLV infected (MOI 3:1); ■, MSV infected (MOI 0.1:1); □, MSV infected (MOI 1:1); ▲, KA 31.

cell growth that preceded saturation density was accompanied by a rapid drop in ODC activity, and basal levels were reached at saturation density. RLV infection had no effect on ODC levels. Transformed KA31 cells had a maximum ODC level approximately twice that of BALB/3T3 cells. Acute MSV infection, at both high and low multiplicities, resulted in a rise of 6–26-fold in maximum ODC activity. The rise commenced at the onset of morphological transformation, and maximum levels coincided with mass transformation of the culture. ODC activity remained elevated during the remainder of the 7-d observation period. When MSV infected cells were trypsinised and reseeded at seven-day intervals (Fig. 2), maximum ODC levels during subsequent passages were about twice as much as uninfected control cells.

Our data demonstrate that a marked rise in ODC activity occurs in cells acutely infected with MSV. This rise is due to the transforming virus component of the MSV complex, as infection with non-transforming MuLV has no effect. The rise in MSV infected cells is not due to an increased rate of cellular division, since most acutely infected cells fail to divide. Rapidly dividing, chronically transformed cells (both virus producing and non-producing) have maximum ODC levels approximately double that of non-transformed parent cells.

The increased ODC levels of virus transformed and other malignant cells may be related to the higher polyamine content of tumour cells and to the increased excretion of polyamines in the urine of cancer patients¹⁴.

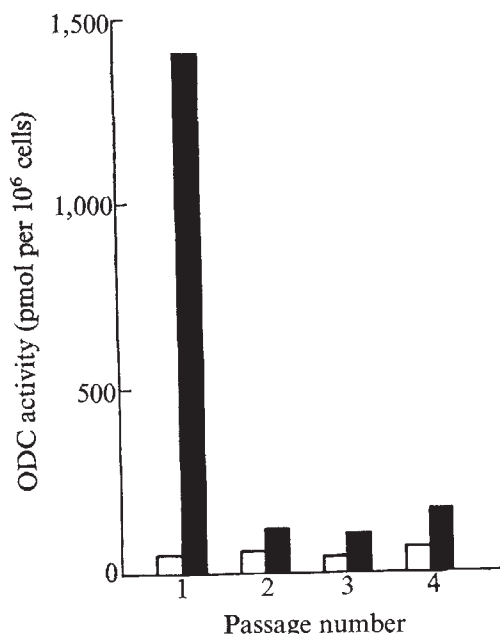


Fig. 2 Maximal ODC levels of MSV BALB/3T3 cells. See Table 1 and Fig. 1. Cells were infected at a MOI of 0.1:1 immediately before seeding at a density of $5 \times 10^3 \text{ cm}^{-2}$. Control and infected cells were trypsinised at 7-d intervals and reseeded at the same density. Cells were collected 4 d after seeding for ODC determinations. Open columns, control cells; solid columns, MSV infected cells.

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Single ionic channels induced in lipid bilayers by polyene antibiotics amphotericin B and nystatine

AMPHOTERICIN B and nystatine increase the permeability of lipid membranes to ions, water and non-electrolytes^{1,2}. Cholesterol (or some other sterols) is a necessary membrane component for the antibiotics to be effective^{1,3}. Permeability to water and non-electrolytes increases linearly with ion

permeability². The molecules of the size of glucose and larger are essentially impermeant. It is concluded^{2,4} that amphotericin B and nystatine form aqueous pores with an effective radius of approximately 4 Å in thin lipid membranes. The changes in membrane conductance connected with the formation of a single pore have, however, not yet been observed. In this paper the properties of individual conductance channels induced in black lipid membranes by amphotericin B and nystatine are described briefly.

Black lipid membranes were formed across a 0.5-mm diameter hole in a 1-ml Teflon cup from *n*-hexane solutions of brain lipids (20 mg ml⁻¹) purified from neutral lipids by acetone extraction. A certain amount of recrystallised cholesterol (usually 1 mg ml⁻¹) was added to the membrane-forming solution. The membrane currents were measured with the Keithley-301 electrometer. The water-soluble sodium salt of amphotericin B or stock solution of nystatine in dimethylsulphoxide (10⁻³ M) were added at equal concentrations to both aqueous solutions buffered with 10 mM potassium phosphate. All the measurements were carried out at pH 6.0 and 22–23 °C. The specific conductance of black lipid membranes in the 2 M KCl solution in the absence of antibiotics was about $2 \times 10^{-9} \text{ mho cm}^{-2}$.

When amphotericin B was added at a concentration of $2\text{--}3 \times 10^{-8} \text{ M}$ to both electrolyte solutions discrete current jumps can be observed on the membranes formed from the heptane-lipid solution with a phospholipid-cholesterol weight ratio of 20:1 (Fig. 1).

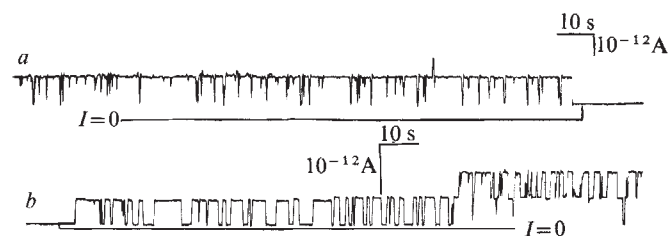


Fig. 1 Fluctuations in membrane current in the presence of $3 \times 10^{-8} \text{ M}$ amphotericin B and 2 M KCl (a), and $1 \times 10^{-7} \text{ M}$ amphotericin B and 2 M KNO₃ (b). Membrane potential 200 mV. Arrows show current through an unmodified membrane. Phospholipid-cholesterol ratio, 20:1.

The probability of the appearance of these conductance jumps (about 6 pmho in 2 M KCl with a membrane potential of 200 mV) depended markedly on the cholesterol content of the membrane-forming solution. With the cholesterol-depleted lipids the membrane conductance did not increase even if amphotericin B at a concentration of $5 \times 10^{-7} \text{ M}$ was added symmetrically to the aqueous solutions. At a phospholipid-cholesterol ratio of 100:1, the current jumps could be observed at 10^{-7} M amphotericin B, whereas at a ratio of 2:1, only a $1\text{--}2 \times 10^{-9} \text{ M}$ concentration of the antibiotic was required. The size of the jumps did not depend on the cholesterol concentration. Being added only to one side of the membrane with a 20:1 ratio of phospholipid-cholesterol, amphotericin B did not increase the membrane conductance even at a concentration of $4 \times 10^{-5} \text{ M}$. In all the experiments described below the phospholipid-cholesterol ratio was 20:1.

The stepwise conductance changes observed seem to be the result of the formation of single ionic channels, each having only two states: one open and one closed. After being formed, the channel exhibits many transitions between the two states. All the transitions shown in Fig. 1a can be related to the same channel because there are no double or other multiple levels characteristic of simultaneous operation of several independent channels. The distribution functions of dwell times for both the open and closed states of an individual channel are single exponentials. The average dwell time in each state does not depend on the membrane potential.

The dependence of a single-channel conductance on KCl

Table 1 Variation in amphotericin B channel parameters with KCl concentration

	KCl (M)				
	0.5	1.0	1.5	2.5	3.5
g (mho $\times 10^{12}$)	2.0 ± 0.4	3.75 ± 0.5	5.5 ± 0.5	6.9 ± 0.5	8.4 ± 0.8
Amph. (M $\times 10^8$)	10	6.5	5	3	2
τ_o (s)	1.9 ± 0.2	—	2.5 ± 0.6	—	4.3 ± 0.5
τ_c (s)	0.14 ± 0.01	—	0.12 ± 0.02	—	0.13 ± 0.02

g , A single-channel conductance measured with membrane potential 200 mV.

Amph., Amphotericin B concentration necessary to obtain a stationary record (about 10 min) in a few channels.

τ_o , Average dwell time of a channel in the open state.

τ_c , In the closed state.

concentration (Table 1) shows saturation. The voltage-current characteristic of a single channel and a multichannel membrane are shown in Fig. 2. The channel conductance increased slightly with membrane potential. In asymmetrical conditions (1 and 3 M KCl), the values of the zero current potentials were the same for the single channel (17 ± 2.0 mV) and for the multichannel membranes (18.5 ± 0.5 mV), within the limits of experimental errors. Transport numbers for the channel ($T_{Cl} = 0.85$ and $T_K = 0.15$) were similar to those found previously for the multichannel membranes in the experiments with low salt concentration¹. Thus, non-ideal anionic permeability of a membrane in the presence of amphotericin B is not the result of simultaneous operation of two types of channels—cationic and anionic—but it is an intrinsic property of each individual channel.

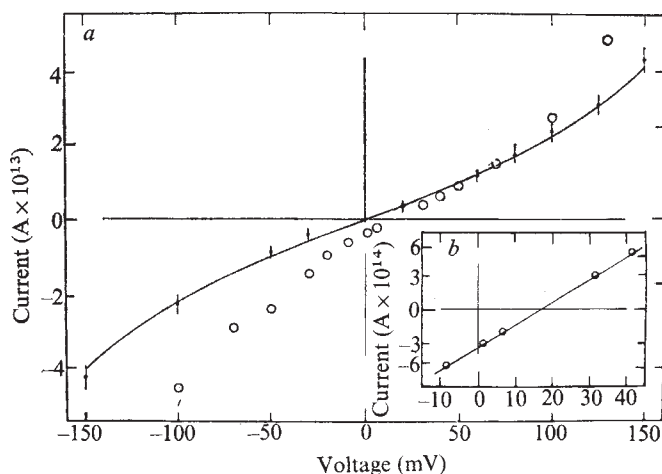


Fig. 2 Current-voltage characteristics of the membrane in the presence of amphotericin B; *a*, points characteristic of a single-channel membrane in a symmetrical solution: 1 M KCl and 6×10^{-8} M amphotericin B; curve, characteristic of the membrane with approximately 200 channels, in a symmetrical solution: 1 M KCl and 2×10^{-7} M amphotericin B. The current scale for the curve was chosen to fit the point at +100 mV. $\circ$, Characteristic of a single-channel membrane separating solutions of 1 M KCl and 3 M KCl; 3×10^{-8} M amphotericin B in both solutions. The sign of the potential is given for the 3 M KCl solution. *b*, Region of characteristic of a single-channel membrane in asymmetrical conditions near the point of zero current potential.

The main feature of amphotericin B channels is the dependence of their formation and properties on the type and concentration of salt. Single channels could be observed in solutions of high concentrations of such salts as KCl, KBr, KF, KNO_3 , CsCl, CsBr, CsF, $CsNO_3$, $RbNO_3$, $CaCl_2$. As a rule these channels have only two states. The jumps of intermediate values, however, were rarely observed, as can be seen in Fig. 3. Table 1 demonstrates that the amphotericin B concentration necessary to obtain a few channels was influenced by salt concentration. Formation of the channels depended on the

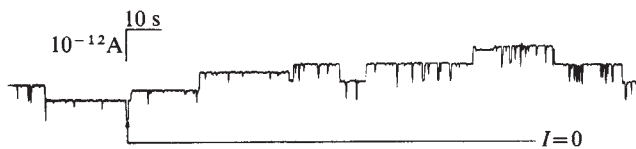


Fig. 3 Current against time record: 5 M CsCl and 2×10^{-6} M amphotericin B. Membrane potential 100 mV.

type of cation as well as anion. If the membrane was formed in solutions of electrolyte with a large cation (2 M acetylcholine chloride) or a large anion size (2 M potassium propionate), the membrane conductance did not increase even at an amphotericin B concentration of 10^{-6} M. In NaCl solutions single channels were observed only at a rather low salt concentration (up to 2 M). In a solution of 2 M NaCl the channel conductance was about 3 pmho. In solutions of higher NaCl or NH_4Cl concentrations (3–4 M), there are only random changes of conductance with irregular jumps of no more than 0.2 pmho.

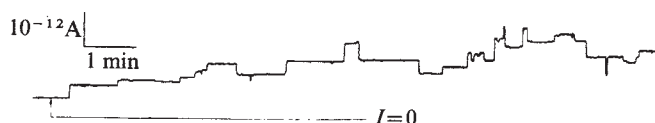


Fig. 4 Current against time record: 3 M KCl and 10^{-7} M nystatine. Membrane potential 200 mV.

One more interesting property of amphotericin B channels is the salt dependence of their gates. Figure 1 shows that the dwell time of the channel in both states at a given voltage were different in KCl and KNO_3 solutions of the same concentration. Moreover the average time which the channel spends in the open state (τ_o) depends on salt concentration, as can be seen in Table 1.

Another polyene antibiotic, nystatine, also induced discrete changes of lipid membrane conductance. In contrast to amphotericin B channels, the presence of smaller jumps and somewhat longer dwell times for the open channels were characteristic of the nystatine channels (Fig. 4).

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Release of novel prostaglandins and thromboxanes after immunological challenge of guinea pig lung

THE ability of lung tissue from various species to synthesise and release parent prostaglandins (PGs) has been well established^{1,2} and metabolic conversions of PGs occur rapidly after very efficient uptake from the circulation^{3,4}. Using an isolated perfusion system to allow uptake of the PG precursor, arachidonic acid

(1- ^{14}C labelled), the sensitised guinea pig lungs released a number of radiolabelled substances following challenge with specific antigen⁵. Our attempts to characterise these substances and prepare sufficient material for biological testing have revealed at least two compounds which have not been previously described and others not previously seen in this release system.

Guinea pigs were sensitised to ovalbumin (100 mg intraperitoneally and subcutaneously) and after 3 weeks were killed and their lungs perfused with Tyrode solution⁶. The sensitised lungs were challenged during perfusion with ovalbumin (5 mg), the perfusate collected for 10 min after challenge and stored at 4 °C under nitrogen. A control group of guinea pigs was also perfused without challenge. The compounds of interest in the perfusate were transferred into organic solvent by adsorbing them on to XAD-2 resin (Rohm and Haas) and eluting with methanol. The concentrated methanol extract was then applied on to a silicic acid column and the various substances removed by stepwise elution with increasing concentration of methanol (0–10%) in chloroform. The eluates were concentrated by vacuum distillation and derivatised for gas liquid chromatography and mass spectrometric analysis (GLC–MS).

The following known PGs and related compounds were identified by GLC–MS following their isolation from sensitised challenged guinea pig lungs; (1) 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT)⁷, (2) the hemiacetal of 8-(1-hydroxy-3-oxopropyl)-9,12-dihydroxy-5,10-heptadecadienoic acid (PHD: thromboxane B_2)^{7,8}, (3) PGE_2 , (4) 15-oxo- PGE_2 , (5) 15-oxo-13,14-dihydro- PGE_2 , (6) $\text{PGF}_{2\alpha}$, (7) 15-oxo- $\text{PGF}_{2\alpha}$, (8) 15-oxo-13,14-dihydro- $\text{PGF}_{2\alpha}$. In addition, two new related compounds were characterised, namely 6-oxo- $\text{PGF}_{1\alpha}$ (compound 1) and 7-[2,4-dihydroxy-6-(3-oxo-1-octanyl)-tetrahydropyran-5-yl]-*cis*-5-heptenoic acid (compound 2). No identifiable levels of these compounds were noted in the control extracts.

The mass spectrum and the source of the major fragments of compound 1 as its methyl ester, methoxime, trimethylsilyl derivative is shown in Fig. 1. Ions at m/e 173 and m/e 199 support the presence of a 15-hydroxy group and a 13,14-double bond⁹. The ion at m/e 217 is typical of the F-prostaglandin ring system⁹. The ion at m/e 187 is formed by a McLafferty rearrangement (not possible for a methoxime substituent at C7) with the hydrogen at C9 and confirms the structure of the α chain. Additional evidence for the location of the oxo group at C6 is provided by the ion at m/e 115.

The structures of the ions at m/e 115 and m/e 187 are supported by the appearance of ions at m/e 118 and m/e 190 in the spectrum of the corresponding trideuteriomethyl ester of compound 1A. Significantly, this spectrum lacks ions at m/e 104 and 176, thereby excluding location of the oxo group in compound 1 at C5. The corresponding benzyl-oxime of compound 1A also exhibits the ion at m/e 115 but in this case the McLafferty rearrangement ion appears at m/e 263. Finally, the spectrum of compound 1 after methylation, borohydride reduction of the oxo group, followed by silylation, exhibits an intense ion at m/e 217. The intensity of this ion reduces by approximately 50% in the corresponding sodium borodeuteride product, and appearance of an ion at m/e 218 is noted. This ion at m/e 218 is consistent with the incorporation of one deuterium atom at C6 and subsequent cleavage in the derivative at the C7, C6 bond, alpha to the newly incorporated trimethylsilyl group. These facts are all consistent with the structure of compound 1 being 6-oxo- $\text{PGF}_{1\alpha}$.

The mass spectrum of compound 2 as its methylester, methoxime, trimethylsilyl derivative, is shown in Fig. 2 with its major fragments. The ion at m/e 174 is typical of the aldehydic unit of the thromboxane system⁷ (this appears at m/e 183 in the spectrum of the *d*-9 trimethylsilyl derivative). The absence of an

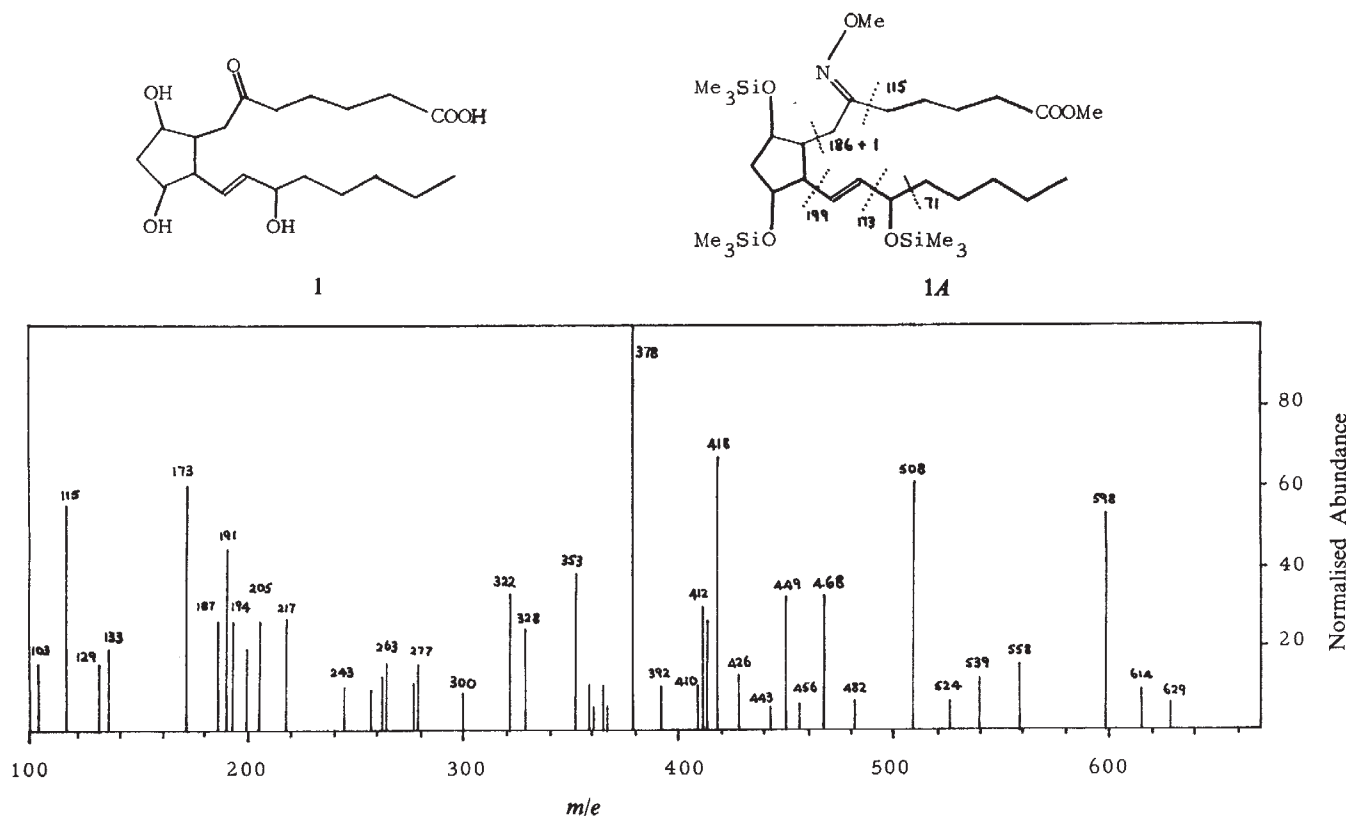


Fig. 1 Mass spectrum of Compound 1 as its methyl ester, methoxime, trimethylsilyl derivative, 1A, showing the source of the major fragments. The principal ions are: m/e 629 (M^+); 614 ($\text{M}-15$)⁺; 598 ($\text{M}-31$)⁺; 558 ($\text{M}-71$)⁺; 539 ($\text{M}-90$)⁺; 508 ($\text{M}-(31+90)$)⁺; 468 ($\text{M}-(71+90)$)⁺; 449 ($\text{M}-(2\times 90)$)⁺; 418 ($\text{M}-(31+90+90)$)⁺; 378 ($\text{M}-(71+90+90)$)⁺; 353 ($\text{M}-(90+186)$)⁺; 328 ($\text{M}-(31+90+90)$)⁺; 217 ($\text{Me}_3\text{SiO}^+=\text{CH}-\text{CH}-\text{OSiMe}_3$); 199 ($\text{CH}=\text{CH}-\text{CH}(\text{OSiMe}_3)\text{C}_5\text{H}_{11}$); 191 ($\text{Me}_3\text{SiO}^+=\text{CH}-\text{OSiMe}_3$); 187 ($\text{CH}_2=\text{C}(\text{NH OMe})(\text{CH}_2)_4\text{COOMe}$)⁺; 173 ($\text{Me}_3\text{SiO}^+=\text{CHC}_5\text{H}_{11}$); 115 ($(\text{CH}_2)_4\text{COOMe}$)⁺.

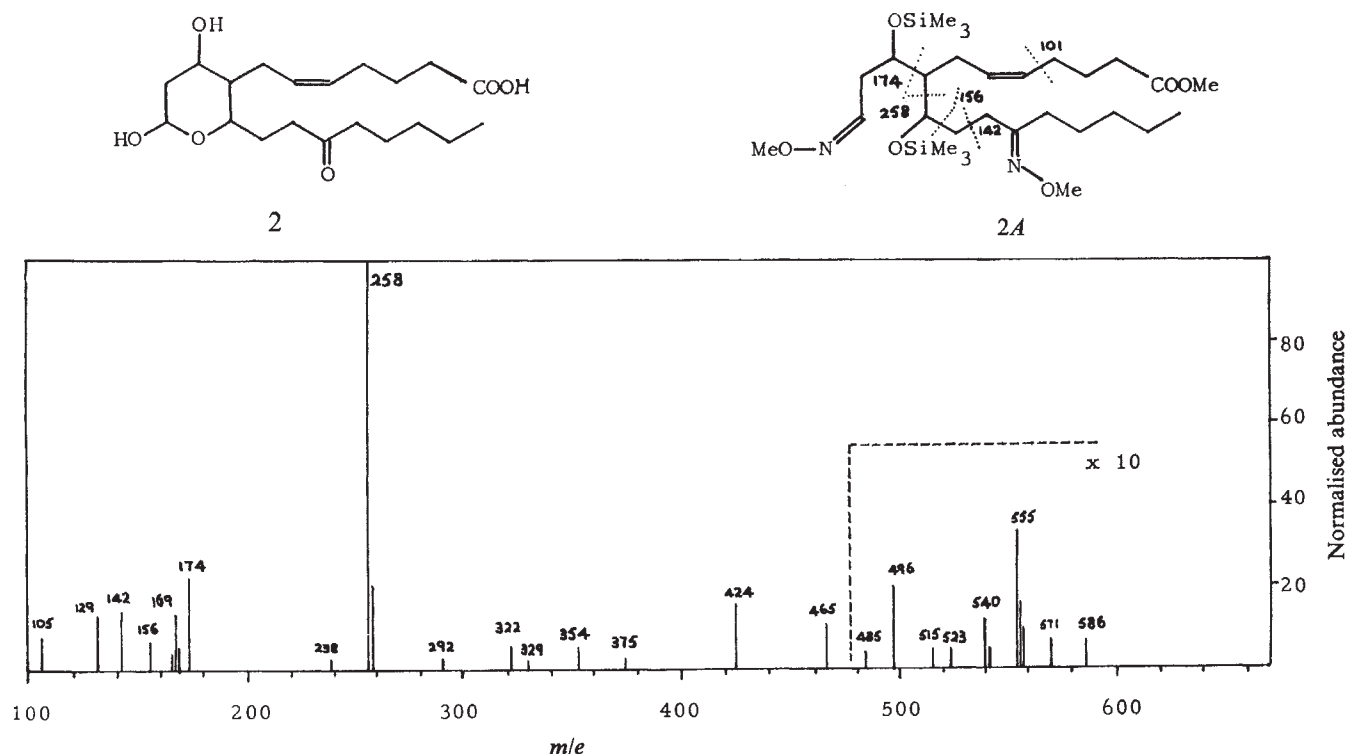


Fig. 2 Mass spectrum of compound 2 as its methyl ester, methoxime, trimethylsilyl derivative 2A, showing the source of the major fragments. The principal ions are: m/e 586 (M^+); 555 ($M-31$) $^+$; 496 ($M-90$) $^+$; 485 ($M-101$) $^+$; 465 ($M-(31+90)$) $^+$; 424 ($M-(90+72)$) $^+$; 375 ($M-(31+90+90)$) $^+$; 354 ($M-(31+90)$) $^+$; 329 ($M-(101+156)$) $^+$; 322 ($M-(90+174)$) $^+$; 258 ($(Me_3SiO=CH(CH_2)_2C(=NOMe)C_5H_{11})^+$); 174 ($(HC(=NOMe)CH_2CH=OSiMe_3)^+$); 156 ($((CH_2)_2C(=NOMe)C_5H_{11})^+$); 142 ($(CH_2C(=NOMe)C_5H_{11})^+$); and ($Me_3SiO=CH-CH_2CN$).

ion at m/e 173 suggests modification of the lower chain from that of thromboxane B_2 . Losses of 156 and 142 and ions at these masses (all noted for the $d-9$ trimethylsilyl derivative) indicate a saturated β chain containing an oxo substituent. The base peak at m/e 258 (267 in the $d-9$ trimethylsilyl derivative) is equivalent to that at m/e 301 in the corresponding thromboxane B_2 derivative⁷. The structure of the top chain is supported by the loss of the 101 fragment (104 in the corresponding trideuteromethylester of 2A). These facts accord with compound 2 having a structure equivalent to that of an oxo-dihydro derivative of thromboxane B_2 .

Table 1 lists the relative amounts of the PGs and thromboxanes observed, along with their chromatographic elution characteristics.

Very little PGE_2 or $PGF_{2\alpha}$ were detected, although there was a significant amount of 15-oxo- $PGF_{2\alpha}$ and 15-oxo-13,14-dihydro- $PGF_{2\alpha}$. A high proportion of the content occurred as compound 2, perhaps indicating a significant formation of thromboxane B_2 . Hamberg¹⁰ described the release of thromboxane B_2 after challenge in a chopped lung system and our data would suggest that *in vivo*, thromboxane B_2 may be metabolised rapidly within the cells after its synthesis and in the lung system, at least, may not exert its full pharmacological action.

The biological activities of compounds 1 and 2 have been compared with those of $PGF_{2\alpha}$ and thromboxane B_2 on a variety of smooth muscle preparations *in vitro*. Compound 2 had no detectable activity, whilst thromboxane B_2 had minimal and variable activity only on respiratory smooth muscle (1/100–1/1000 that of $PGF_{2\alpha}$).

Compound 1, however, had considerable biological activity, being 1/3 as active as $PGF_{2\alpha}$ on respiratory smooth muscle and having 1/5 to 1/100 the activity on a variety of intestinal smooth muscle preparations. Compound 1 occurs as approximately 11% of the total PGs released on challenge ($PGF_{2\alpha}$ 0.6%) and probably accounts for a significant proportion of the PG spasmogenic activity.

Only three of the compounds listed in Table 1 have significant spasmogenic activity: $PGF_{2\alpha}$, 15-oxo- $PGF_{2\alpha}$ (ref. 11) and 6-oxo- $PGF_{1\alpha}$ and the activity of the last two seems to be reasonably selective for respiratory smooth muscle. It would seem, therefore, that the identification of this novel PG (compound 1) is of considerable relevance to the immunological reaction in guinea pigs and may relate to the symptomology of human asthma.

Although there is no evidence in the present study for metabolic conversion of thromboxane B_2 to compound 2, by inference from the metabolism of the parent PGs, it seems likely

Table 1 Chromatographic characteristics and relative amounts of compounds isolated after immunological challenge of guinea pig lung

Compound	Fraction no.*	C-value†	Approximate % of total‡
15-oxo PGE_2	3	24.4§	Trace
15-oxo-13,14-dihydro PGE_2	3	24.3§	Trace
15-oxo-13,14-dihydro $PGF_{2\alpha}$	3	24.4	3
PGE_2	3,4	24.5§	1
Compound 2	4,5	25.3	42
15-oxo- $PGF_{2\alpha}$	4	24.5	6
Thromboxane B_2	6,7,8	25.1	30
$PGF_{2\alpha}$	7	24.0	0.6
Compound 1	7,8	25.5	11
Other unidentified substances with PG-like mass spectral characteristics	—	—	6

*% MeOH in $CHCl_3$ on silicic acid, each fraction being 20 ml total volume.

†Retention time relative to fatty acid esters on 1.2% SE 30 at 230 °C for methylated, methoximated, silylated compounds. Compound 1 is not resolved into doublet except as benzyl-oxime derivative.

‡Calculation based on 3 groups of pooled perfusates each derived from 200 guinea pigs. There was less than 10% variation in the percentages quoted from each group. GLC analysis indicated that the total PG and thromboxane content is 1.3–1.8 μ g per guinea pig.

§Major geometric isomer.

that this is its probable mode of formation. The activity of the enzymes responsible for these metabolic reactions in asthmatic individuals could, therefore, be of considerable importance in an analysis of the role of PGs and thromboxanes in asthma.

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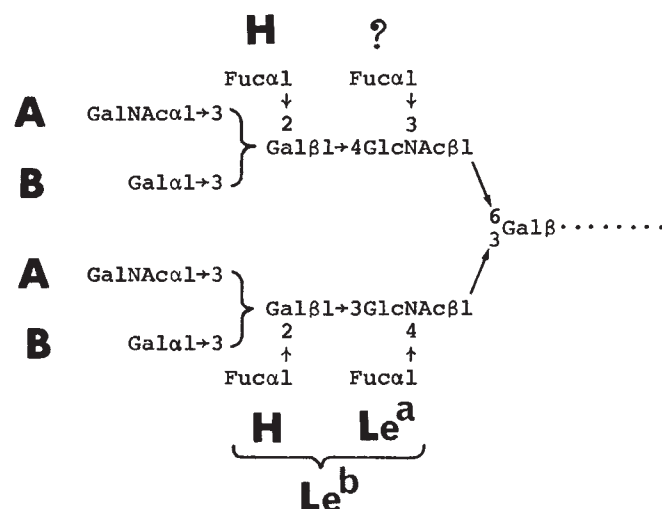
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ABO blood group determinants with branched cores

ALTHOUGH the biochemical basis of ABO and Lewis blood types in man has on the whole been elucidated¹, more detailed information is needed about the carbohydrate structures of the A and B subgroups². But investigation has been limited by the complexity of the sugar chains of these antigens³. Information can, however, be obtained from the study of human milk oligosaccharides, which are synthesised by a series of glycosyl transferases which mediate the formation of glycoproteins, especially those of blood group substances¹. Our studies have shown that, contrary to the composite structures of ABO and Lewis blood group determinants described by Lloyd and Kabat³ (Fig. 1), the H determinant is located only on the type 1 carbohydrate chain (Galβ1→GlcNAc....) of the branched sugar grouping.

Lacto-*N*-hexaose, a branched oligosaccharide with the same structure as the distal portion of the backbone carbohydrate

Fig. 1 Composite structure of the blood group determinants proposed by Lloyd *et al.*³.



chain of blood group substances, and its fucosyl derivatives have been found in human milk^{4,5}. We have isolated three of them which have the Fucα1→2Gal grouping and elucidated their structures (Fig. 2). Details of structural studies will be published elsewhere.

A survey of these structures suggested that the Fucα1→2Gal grouping is limited on the type 1 chain of the branched portion of the backbone, in contrast to the widely accepted idea that the grouping occurs both on type 1 and type 2 chains of the branched core⁶. To verify this concept, the possible occurrence of even a minor isomeric component that has the Fucα1→2Gal grouping on the type 2 chain had to be eliminated. An *N*-acetylgalactosaminyl transferase (A enzyme) found in the milk of women with blood type A transfers *N*-acetylgalactosamine from UDP-GalNAc to the C3 position of the galactose residue of both Fucα1→2Galβ1→3GlcNAc.... and Fucα1→2Galβ1→4GlcNAc.... groupings^{7,8}. With UDP-*N*-¹⁴C-acetylgalactosamine as a donor, this enzyme can be used to detect specifically the H determinants with type 1 and type 2 chain backbone⁹.

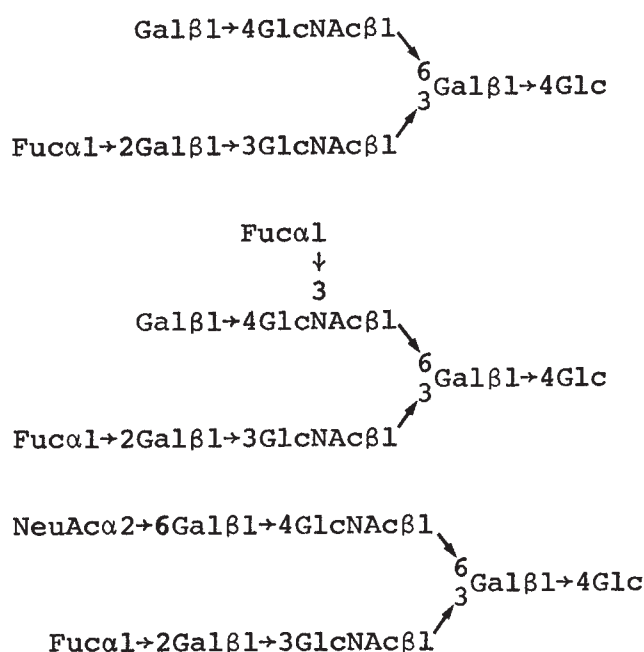


Fig. 2 Structures of three lacto-*N*-hexaose derivatives with blood group H determinant.

An endo-β-galactosidase, purified in our laboratory from *Diplococcus pneumoniae*, hydrolyses the blood group A determinant of type 2 chain but not of type 1 chain¹⁰. A combination of these two enzymes thus provides a sensitive method to discriminate type 1 and type 2 H determinants in heterosaccharide chains as indicated in Fig. 3.

N-2, a mixture of monofucosyllacto-*N*-hexaoses, and N-3, a mixture of difucosyllacto-*N*-hexaoses, were isolated from milk of four secretor individuals as before¹¹. Each oligosaccharide was incubated with UDP-*N*-¹⁴C-acetylgalactosamine and A enzyme¹², and the products were analysed by paper chromatography. A radioactive octaose was detected in all the incubation mixtures of N-2 fractions and a nonaose in all the mixtures of N-3 fractions (Table 1). When the N-2 and N-3 fractions were incubated with 20 μg of *Bacillus fulminans* α-L-fucosidase¹³, which cleaves only the Fucα1→2Gal linkage, no radioactive products were detected. We conclude that the *N*-acetylgalactosamine incorporated into oligosaccharides was catalysed by the A enzyme.

The radioactive octaose and nonaose were eluted from the paper chromatogram, incubated with endo-β-galactosidase, and the release of GalN-¹⁴C-Acα1→3Gal2←1αFuc was determined

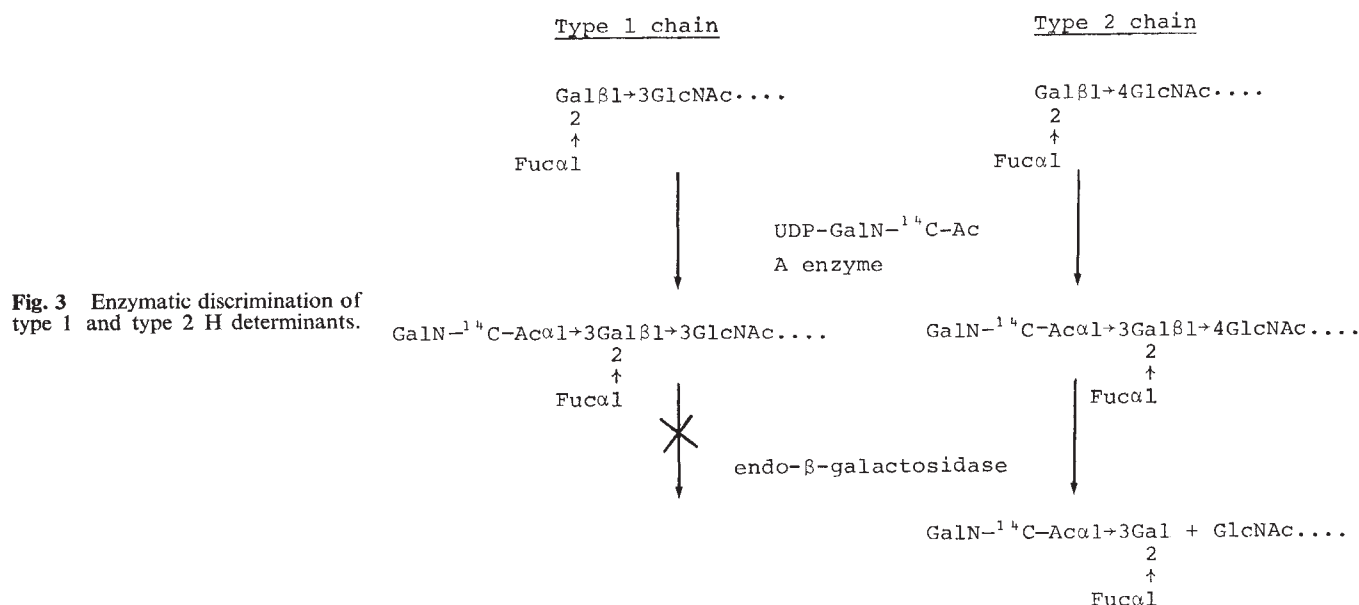


Fig. 3 Enzymatic discrimination of type 1 and type 2 H determinants.

as described in the legend of Table 1. No radioactive trisaccharide was observed. Lacto-*N*-fucopentaose I and Fucα1→2Galβ1→4GlcNAcβ1→3hexene-1,2,5,6-tetrol as standards of

Table 1 Incorporation of *N*-¹⁴C-acetyl galactosamine into oligosaccharides catalysed by A enzyme and their degradation by endo-β-galactosidase

Oligosaccharides	GalN- ¹⁴ C-Ac incorporated (c.p.m. × 10 ⁻³)	Counts released as trisaccharide (c.p.m. × 10 ⁻³)
N-2 fraction		
1	2.1	0
2	1.9	0
3	0.8	0
4	0.9	0
N-3 fraction		
1	4.8	0
2	2.9	0
3	3.2	0
4	1.8	0
Lacto- <i>N</i> -fucopentaose I	7.4	0
Fucα1→2Galβ1→4GlcNAcβ1→3 hexene-1, 2, 5, 6-tetrol	6.8	6.7

A sample (50 μg) of each sugar was mixed with 300 U of purified A enzyme, 20 nmol (0.1 μCi) of UDP-*N*-¹⁴C-acetyl galactosamine and 50 μl of 0.05 M Tris-HCl buffer, pH 7.5, containing 0.02 M MnCl₂. The A enzyme was obtained from the milk of an A₁ individual. After addition of a drop of toluene, the mixtures were incubated at 37 °C for 42 h. The reaction mixtures were then passed through a small column (0.5 × 4 cm) containing a mixed bed of BioRad AG-3 (OH⁻) and BioRad AG-50 (H⁺), and washed with five bed volumes of distilled water. Eluate and washings were combined, spotted on Whatman No. 3MM paper as a 3-cm band and subjected to descending paper chromatography using ethylacetate-pyridine-acetic acid-water (5:5:1:3) as a solvent for 14 d. Radioautoscanning revealed a single radioactive octasaccharide and a single radioactive nonasaccharide in the incubation mixtures of N-2 and N-3 fractions, respectively. Radioactive GalNAcα1→3Gal(2←1αFuc)β1→3GlcNAcβ1→3Galβ1→4Glc and GalNAcα1→3Gal(2←1αFuc)β1→4GlcNAcβ1→3hexene-1, 2, 5, 6-tetrol were detected as the sole reaction products in the incubation mixtures of lacto-*N*-fucopentaose I and Fucα1→2Galβ1→4GlcNAcβ1→3hexene-tetrol, respectively. These radioactive oligosaccharides were recovered from the paper chromatograms by elution with water, and the radioactivities were determined by a Packard Tri-Carb liquid scintillation spectrophotometer, after mixing aliquots of each sample with scintillation cocktail. The remaining samples were incubated with 1.0 mU of purified endo-β-galactosidase for 24 h. The incubation mixtures were spotted on Whatman No. 3MM paper, and subjected to descending paper chromatography using ethylacetate-pyridine-water (12:5:4) as a solvent for 20 h. The areas corresponding to authentic GalNAcα1→3Gal(2←1αFuc) (ref. 9) were cut out from the chromatogram, placed in scintillation vials, and the radioactivities were determined after incubating the paper pieces with 1 ml of water and adding 7 ml of scintillation fluid.

type 1 and type 2 H determinants, respectively, were treated in the same way. Similar levels of *N*-acetyl galactosamine were incorporated into both oligosaccharides by incubation with A enzyme and UDP-*N*-¹⁴C-acetyl galactosamine (Table 1). The activity incorporated into Fucα1→2Galβ1→4GlcNAcβ1→3hexene-1,2,5,6-tetrol was recovered completely as GalNAcα1→3Gal(2←1αFuc) by the subsequent endo-β-galactosidase digestion, while that of lacto-*N*-fucopentaose I remained unhydrolysed (Table 1). These results confirm that the Fucα1→2Gal groupings of N-2 and N-3 fractions are located only on the type 1 chain of lacto-*N*-hexaose. The limited location of the Fucα1→2Gal grouping may be the reflection of the specificity of the *H* gene-associated α-2-L-fucosyltransferase. This enzyme adds α-fucosyl residues at the C-2 position of the non-reducing terminal galactose units of both type 1 and type 2 groupings when these are present on unbranched sugar chains^{14,15}. This enzyme, however, may not be able to add a fucosyl residue at the Galβ1→4GlcNAc grouping of the branched core.

The Galβ1→4GlcNAcβ1→6Gal... grouping has been shown to be one of the I blood group determinants^{16,17}. Absence of the H blood group determinant on this grouping means that the ABO blood group system does not interfere with the phenotypic expression of the *I* gene, and that the I antigen may be able to coexist with A, B or H antigen on the same branched heterosaccharide core.

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Lead competitively inhibits calcium-dependent synaptic transmission in the bullfrog sympathetic ganglion

MANY polyvalent cations have been used in studies of synaptic transmission either as tools for gaining a better understanding of synaptic processes or, in their own right, as potentially toxic substances¹⁻⁷. Inorganic lead although long known to have neurotoxic effects, has not been studied extensively at the synaptic level until recently. Kostial and Vouk⁸ found that lead blocked transmission in the perfused superior cervical ganglion of the cat *in vitro* by reducing the amount of acetylcholine released from presynaptic nerve terminals without affecting the response of ganglion cells to applied acetylcholine. The addition of excess calcium to the perfusion solution restored acetylcholine output and relieved the lead block. Manalis and Cooper⁹, working with the frog sciatic nerve-sartorius muscle preparation *in vitro*, showed that lead depresses phasic transmitter release evoked by nerve stimulation and increases spontaneous release as evidenced by an increase in frequency of miniature endplate potentials. Lead had only a weak, curare-like effect on the postsynaptic response to applied acetylcholine. In this paper we show, using electrophysiological techniques, that PbCl_2 blocks synaptic transmission presynaptically by competitive inhibition of calcium. In support of this data, we further show, using ^{45}Ca , that lead in fact does reduce the uptake of calcium by preganglionic nerve terminals.

In all experiments the 9th or 10th sympathetic ganglion was removed from the bullfrog (*Rana catesbeiana*) along with several cm of preganglionic trunk and postganglionic ramus and connecting spinal nerve. After removal of the connective tissue sheath, the ganglion was placed in a sucrose gap chamber similar to that described by Koketsu and Yamamoto¹⁰. This chamber allowed for the selective perfusion of the ganglion with normal or modified Ringer's solutions. Supramaximal stimuli were applied at a rate of 1 s^{-1} . Control ganglionic compound action potential was measured after 30 min of stabilisation in normal Ringer. Then the ganglion was perfused for 30 min with Ringer's solution containing lowered calcium, added lead, or both. Control and experimental periods were alternated in this way for the duration of an experiment.

Data for 4 concentrations of lead at several calcium concentrations are presented in Fig. 1. Figure 1a shows that the log of ganglionic response amplitude is a positive linear function of the log of calcium concentration between 0.2 and 1.0 mM. Lead shifts the response to the right with no appreciable change in slope. The slopes of the log-log curves are similar to those obtained for cobalt and magnesium at the neuromuscular junction^{1,11}. The same data replotted on modified Lineweaver-Burk coordinates as described by Dodge and Rahamiandmoff¹¹ are shown in Fig. 1b. These curves intercept with the ordinate at the same point, indicating a competitive antagonism of calcium by lead. The potency of lead as a synaptic blocking agent is great when compared with magnesium, cobalt, and manganese, all of which have been shown to interfere with synaptic transmission at the frog neuromuscular junction through a competitive antagonism of calcium. Cobalt and manganese are 20 times^{1,2} and lead is 1,000 times⁹ more potent than Mg at the frog neuromuscular junction. Our data reported here are in general agreement since lead is at least 200 times more effective than magnesium in blocking ganglionic transmission³.

Several other parameters were examined to ascertain whether the sole effect of lead at these concentrations was through an antagonism of the action of calcium at presynaptic nerve terminals. Axon conduction amplitude and velocity in the preganglionic nerve trunk were measured before and after exposure to lead in a concentration which completely blocked ganglionic transmission. No changes were seen. Presynaptic

spike amplitude was measured by the method of Dunant¹². Again, lead had no appreciable effect. Ganglionic response to bath-applied acetylcholine as described by Koketsu and Yamamoto¹⁰ was used as a test of postsynaptic receptor function. Only a 5% decrease in response was observed at levels of lead which completely block synaptic transmission.

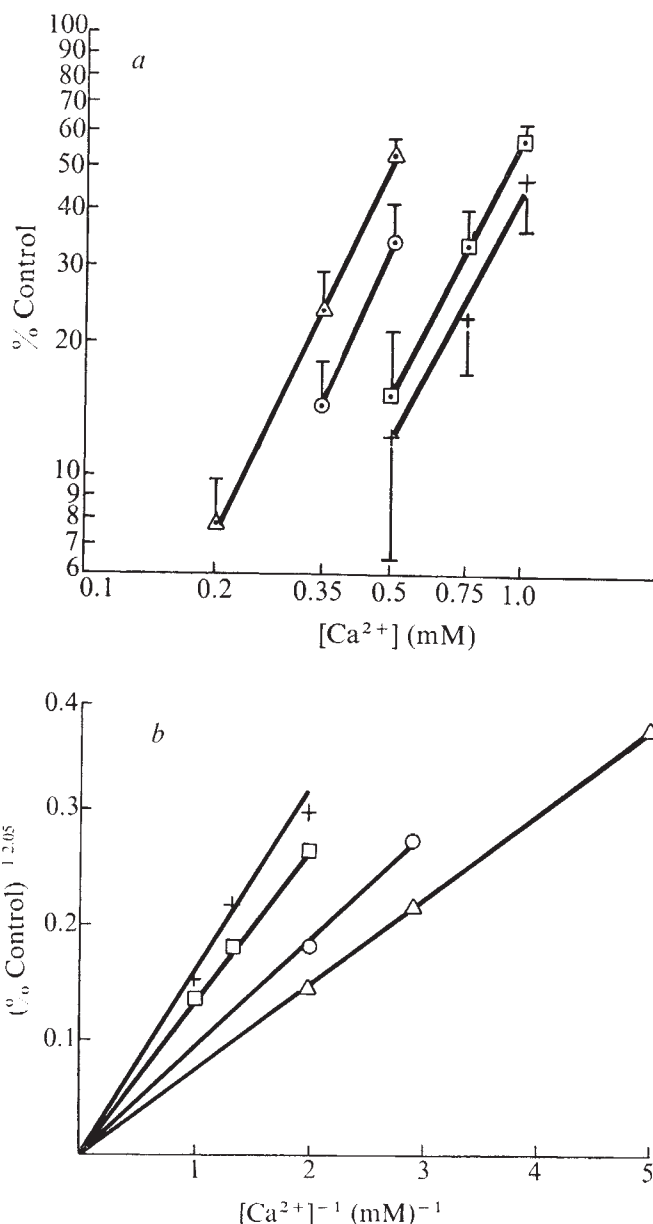


Fig. 1 a, A log-log plot of postganglionic response amplitude against Ca^{2+} concentration for 0, 5, 10, and 20 μM Pb^{2+} . The average slope of the lines is 2.05. Each point is the average of at least 5 determinations. The standard error of the mean is shown with each point. b, The same data plotted on modified Lineweaver-Burk coordinates. Control Ringer contained 111 mM NaCl, 2.5 mM KCl, 2.0 mM CaCl and 4 mM Tris buffer. All straight lines were determined by least-squares regression. Concentration of Pb^{2+} in modified Ringer's solution: Δ , 0 μM ; $\circ$, 5 μM ; $\square$, 10 μM ; +, 20 μM .

From the data presented above we infer that lead competitively inhibits calcium-mediated transmitter release from presynaptic nerve terminals. Additional support of this conclusion was obtained in a series of experiments in which the effects of lead on the uptake of ^{45}Ca by presynaptic nerve terminals were examined. The method of Blaustein was used with several minor modifications¹³. ^{45}Ca was added to a bath containing a pair of ganglia. One member of the pair was stimulated

supramaximally for 20 min at a rate of 6 stimuli s^{-1} . After rinsing, weighing, and digestion, the amount of ^{45}Ca taken into the ganglia was measured by conventional scintillation counting.

Figure 2 shows that stimulation of the preganglionic nerve resulted in a threefold increase in ^{45}Ca uptake. Antidromically stimulated ganglia took up no more ^{45}Ca than unstimulated ganglia indicating that the activity of ganglion cells and postganglionic axons did not contribute to the ^{45}Ca uptake. Furthermore, ganglia blocked by (+)-tubocurarine took up quantities of ^{45}Ca comparable to the uptake of unblocked preparations suggesting that postsynaptic receptor activity was not contributory. The ^{45}Ca uptake of stimulated lead-blocked ganglia was however, reduced to 9% of control, indicating that lead strongly blocks the uptake of calcium normally associated with the arrival of action potentials at the presynaptic nerve terminals. This is in agreement with the data and the conclusion derived from the electrophysiological studies. In addition, lead reduced the ^{45}Ca uptake of unstimulated ganglia to 16% of unstimulated control indicating an effect of lead on calcium exchange which is not related to the presence of an action potential.

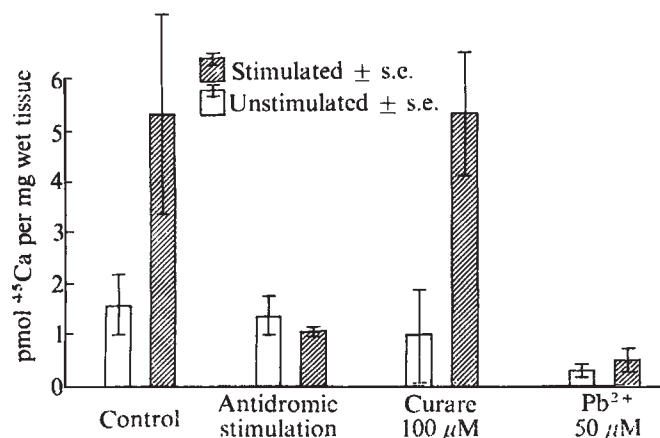


Fig. 2 The amount of ^{45}Ca taken up by ganglia under the following conditions: *a*, unstimulated ganglia and ganglia activated by preganglionic nerve stimulation, both in normal Ringer's solution; *b*, unstimulated ganglia and ganglia activated by stimulation of the postsynaptic nerve, both in normal Ringer's solution; *c*, unstimulated ganglia and preganglionically stimulated ganglia in Ringer's solution containing 100 mM (+)-tubocurarine; *d*, unstimulated and preganglionically-stimulated ganglia in Ringer's solution containing 50 μ M Pb $^{2+}$. 50 μ M Pb $^{2+}$ blocks 100% of the postganglionic response. Modification of Blaustein's method was as follows: 5 μ Ci of ^{45}Ca in 50 μ l HOH were added to a bath containing 2.5 ml of Ringer's solution. After stimulation, the pre- and postganglionic nerves were removed and the ganglia blotted on filter paper. Wet weights were then taken. Digestion was done in 0.5 ml of 10% tetramethyl ammonium hydroxide heated to 90 $^{\circ}C$ for 20 min. 5 ml of Bray's solution were then added for conventional scintillation counting.

From these experiments we conclude that lead blocks synaptic transmission in the sympathetic ganglion by competitive antagonism of spike-evoked entry of calcium into presynaptic nerve terminals and a subsequent reduction of transmitter release. It is not possible to say whether such a mechanism contributes significantly to the central or peripheral nervous system dysfunction seen in acute or chronic lead poisoning. Carroll *et al.*¹⁴ have, however, shown that K-induced release of acetylcholine from brain slices of mice exposed to lead during development is reduced and is also dependent on the presence of calcium in the medium. Since the presently accepted upper limits of normal, "safe", blood lead levels of human children and adults are 2 μ M and 4 μ M respectively¹⁵, it may well be that the mechanism described here is a factor in chronic lead poisoning.

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Type of sensory nerve fibre sprouting to form a neuroma

WHEN a peripheral nerve is cut across, the axons in the central end send out sprouts. If these sprouts fail to reach the distal part of the cut nerve they form a tangled mass called a neuroma¹. Neuromas which occur in man after amputation or after failure of regeneration of a damaged nerve are always tender on pressure and are sometimes the source of ongoing pain². When experimental neuromas are stimulated, it is known that a delayed volley of nerve impulses arrives at the central nervous system³ and it was assumed that the delay was occurring in the fine sprouts. Now that we have examined single fibres originating from a neuroma, we find that this conclusion was wrong and that there is a much more surprising explanation: only the small diameter, slowly conducting axons within the peripheral nerve seem to send sprouts into the neuroma and the delay in arrival of the afferent volley is primarily due to the fact that it runs only in slowly conducting fibres. This selective outgrowth of small fibres may be of considerable practical interest. These fibres include those which normally carry signals from injured tissue. In the normal situation, however, injury detection signals are accompanied by impulses in large diameter nerve fibres which decrease the central effect of the injury signals. The neuroma, as a pure culture of small fibres, may generate pain producing impulses³ where the pain has an intensity and peculiarly unpleasant quality due to the absence of the normal modulating impulses⁴ in the larger fibres which have failed to regenerate.

The experiments were carried out on 70 adult male albino rats, Sabra strain, weighing more than 300 g at the time of nerve section. Under ether anaesthesia, the sciatic nerve of one leg was transected and the central stump was drawn by a strand of epineurium into a polythene tube so that the nerve fitted snugly without compression. The end of the tube was usually capped with bone wax. Some nerves were studied in an acute preparation immediately after cutting, and others were studied 1 d to 6 months after section. For this examination the animals were anaesthetised with urethane or Nembutal, paralysed with Flaxedil, and had the lumbo-sacral spinal cord exposed. To record compound action potentials, a low lumbar dorsal rootlet originating in the neuroma was cut centrally and placed on a pair of Ag-AgCl recording hooks. For single units, fine filaments, about 20 μ m in diameter and 1-2 mm long were stripped from the end of the root, placed on a single hook, and recordings made with reference to a nearby indifferent electrode. The sciatic nerve was exposed and the neuroma freed from its tube.

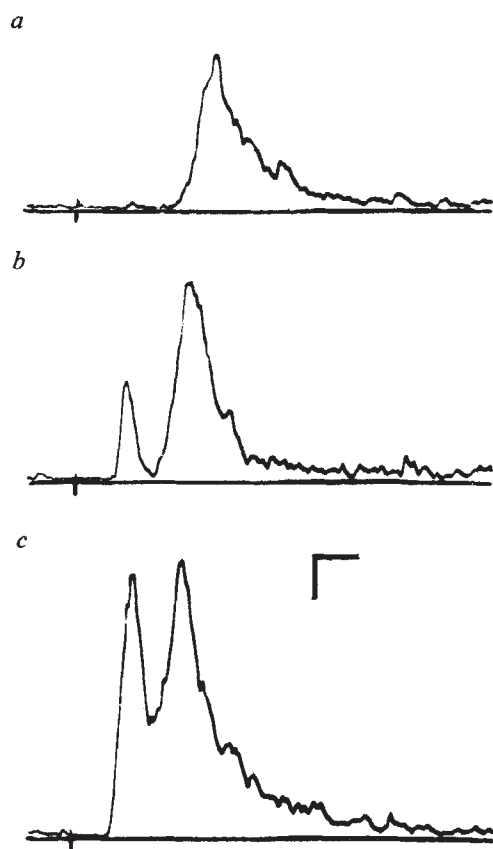


Fig. 1 Compound action potentials recorded on an L5 dorsal root following stimulation of a sciatic nerve neuroma (*a*), a point on the proximal edge of the neuroma (*b*), and a region of relatively intact sciatic nerve (*c*). Recordings were made 52 d after the sciatic nerve had been cut. The neuroma was 4 mm long. Stimulus points for *a*, *b* and *c* were 2.5, 7.5 and 12.5 mm central to the distal end of the neuroma. A cathodal square wave pulse (2.0 mA, 0.05 ms) was delivered at the point indicated in each sweep. The calibration marks are 1 ms and 200 μ V negative upwards. The results show that only a slowly conducting wave was produced from the neuroma while a bimodal compound action potential was evoked by stimulation only slightly proximally on the nerve.

The exposed surface of the neuroma and nerve was then stimulated at selected points by silver hook or mono- or bipolar fine tungsten electrodes delivering square wave pulses of 0.01–0.5 ms duration from a constant current source.

By one week after the nerve had been cut, an obvious swelling appeared on the end. When this was stimulated at a current adequate to stimulate intact nerve, no response was recorded. If the stimulus was increased, a compound action potential appeared on the root after an unusually long delay. An example is shown in Fig. 1*a* where the nerve was examined 52 d after section and the stimulus applied midway along the 4-mm neuroma. Using the same intensity of stimulus, compound action potentials were also evoked by stimulation at points 5.0 mm (Fig. 1*b*) and 10.0 mm (Fig. 1*c*) proximally along the nerve. It will be seen that the compound action potential evoked from 10.0 mm proximal to the neuroma has two peaks and arrives in 0.8 ms. The wave evoked from the neuroma itself, only 10 mm distally, however, takes an extra 1.4 ms to arrive. Similar results were seen in all nerves tested from a few days to 6 months after section. The extent of the delay depended on the survival time with delays increasing for about 3 weeks. Thereafter, little further slowing occurred. The question of whether this delay is due entirely to slowing of spike conduction within the neuroma or to the possibility that only slowly conducting parent fibres penetrate the neuroma was only settled with certainty by single unit studies.

Single fibres originating from the neuromas were examined by

recording from fine dorsal root filaments and by stimulating at two locations, one (S_1) on the intact nerve, usually about 20 mm from the neuroma, and one (S_2) on the neuroma itself (Fig. 2, top). The two stimulus locations allowed the single unit nature of the spike to be defined by the collision technique. First a unit driven from the neuroma was identified and its latency measured and conduction velocity (S_2 velocity) calculated. Then a shock was applied to the proximal stimulating electrode (S_1) 0.5–10 ms before the neuroma stimulus, and its intensity raised gradually. Suddenly the spike originating in the neuroma vanishes and is replaced by an earlier identical spike. The proximal stimulus has generated an orthodromic spike which is recorded and an antidromic spike which has collided with the spike originating from the neuroma, abolishing it (Fig. 2). The latency of the spike from the S_1 stimulus can now be measured and the conduction velocity of the fibre from the S_1 position to the recording electrode (S_1 velocity) calculated. This technique allowed single myelinated and unmyelinated afferents to be identified and

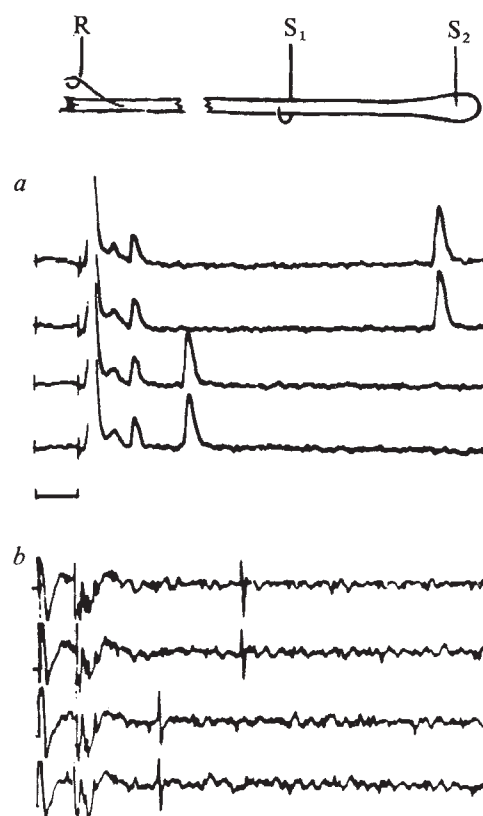


Fig. 2 Single units identified by the collision technique. The recording and stimulation arrangement is shown in the diagram at top. *a*, Top sweep, S_1 stimulus is delivered at the beginning of the sweep and evokes a volley of spikes (compound action potential) on the dorsal root filament from 1–2 ms after the stimulus. The S_2 stimulus is given 1 ms after the S_1 stimulus and it evokes a single spike with a latency of 7.9 ms seen on the right of the sweep. The stimulus current on S_1 is increased in the second sweep but is still below threshold for the single unit. In the third and fourth sweeps, the strength of the S_1 stimulus has reached the unit's threshold and a spike now appears 3.3 ms after the S_1 stimulus. This spike has the same height and slope as the one evoked by the S_2 stimulus in the sweeps above. When the spike is produced by the S_1 stimulus it collides with the spike produced distally in the neuroma (S_2) so that the spike produced from S_2 cannot travel to the recording root. This proves that the action potential from the two stimulus points occupied the same fibre. The overall conduction velocity from S_1 was 24.8 m s^{-1} and from S_2 was 11.9 m s^{-1} . *b*, Using the same procedure as in *a*, a single unit with a lower conduction velocity was identified. Here the S_1 – S_2 time difference was 10 ms and the overall conduction velocity was 2.2 m s^{-1} from the S_1 stimulus point and 2.2 m s^{-1} from the S_2 point. The time mark is 1 ms for *a* and 10 ms for *b*.

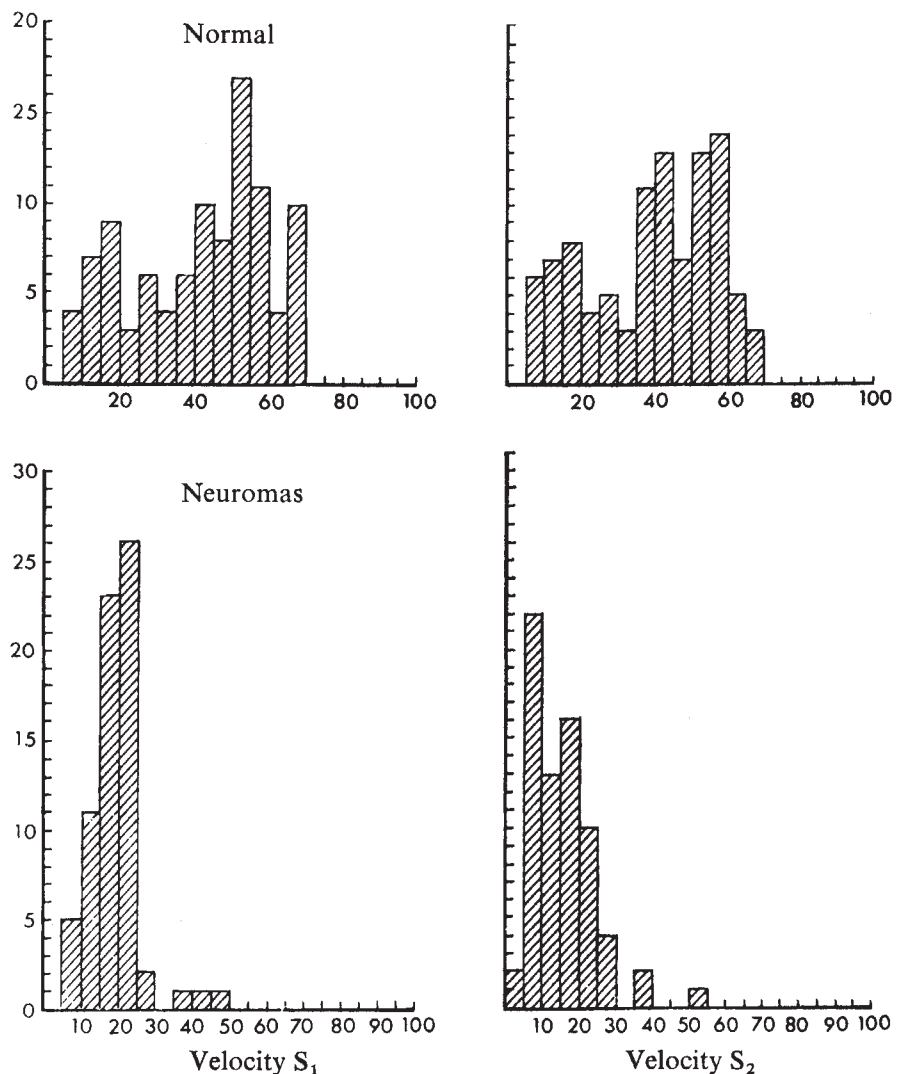


Fig. 3 Histogram of conduction velocities of 99 successively encountered single fibres in normal nerves and of 70 originating from neuromas of 50–84 d standing. The recording and stimulus situation is shown in the diagram in Fig. 2. For normal fibres, the sciatic nerve was intact and the stimulus points were at the standard positions used in the neuroma animals. The conduction velocity of each single fibre was calculated, using the collision method, both for the S₁ stimulus position on proximal nerve and for the S₂ position at the neuroma. It will be seen that virtually all impulses elicited by electrical stimulation of the neuroma run in fibres which have a low conduction velocity in the proximal nerve. Only sprouts of slowly conducting fibres appear to contribute to the neuroma. The velocities from S₂ are slower than those from S₁ because there has been a slowing of the impulses in the fine sprouts within the neuroma.

allowed the calculation of conduction velocities both for the parent fibre alone and its extension into the neuroma.

In a series of unoperated rats, and rats with sciatic neuromas, this was done for every unit encountered from the S₂ stimulus position that was sufficiently well isolated to allow for unequivocal determination of its velocity also from the S₁ position. The histogram of S₂ conduction velocities in normal nerves (Fig. 3, upper right) shows a distribution ranging from 5 m s⁻¹ up to 80 m s⁻¹ with the expected bimodal distribution. The histogram for S₁ velocities of these same fibres is very similar (Fig. 3, upper left) since normal fibres have relatively uniform velocities along most of their course. The histogram of conduction velocities from the neuroma shows, however, that almost all the units sampled from neuromas had slow overall velocities (Fig. 3, lower right) and furthermore their parent fibres in the intact part of the nerve were also slow (Fig. 3, lower left). In addition to the small myelinated fibres whose sprouts make up the neuroma, a number of unmyelinated parent fibres were observed which extended into the neuroma. These fibres, which may have a role in pain along with the small myelinated fibres, will not be discussed further here. They were not included in the histograms because they were specifically sought and not randomly encountered as was the rest of the sample.

Most of the fibres from neuromas had overall (S₂) conduction velocities somewhat slower than the velocities of their parent fibres (Figs 2a and 3, bottom). This difference undoubtedly represents a slowing of the impulses in the tangled fine sprouts within the neuroma. In a few fibres one S₂ response latency was observed when near-threshold stimulating currents were used, but when the current was increased the unit's response latency

suddenly jumped to a smaller value. Apparently, the stimulus had now become suprathreshold at a slightly more proximal position on the sprout. For each spike initiation point within the neuroma, however, the parent fibre velocity measured at S₁ was identical. It has been reported that there is a slight shrinkage of myelinated fibres proximal to a nerve section⁵, a phenomenon reflected in the slight slowing of the fast component of the compound action potential elicited from positions proximal to neuromas as compared with an intact nerve. This change however, is quite inadequate to explain the marked low velocity of the parent fibres of neuroma sprouts.

These data indicate that electrical stimulation of neuromas formed on the transected sciatic nerve activates slowly conducting myelinated (A-delta) but not more rapidly conducting myelinated fibres. This presumably means that only small fibres sprout to form a neuroma. Alternatively, it may be that large fibres also emit sprouts but that orthodromic conduction along them is blocked, perhaps because the fine sprout is unable sufficiently to depolarise the large parent axon⁶.

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Swimming in the sea anemone *Stomphia coccinea* triggered by a slow conduction system

THERE has been great interest in the sea anemone *Stomphia coccinea* since its remarkable swimming response to the starfish *Dermasterias imbricata* was first discovered¹. On contact with *Dermasterias*, this anemone rapidly detaches from the substrate and undergoes a series of swimming movements involving flexions of the column. The starfish *Hippasteria spinosa*² and a nudibranch, *Aeolidia papillosa*³ also evoke swimming. Previous attempts to analyse this response physiologically were based on indirect observations of swimming induced by electrical stimulation. There were difficulties in explaining the coordination of the swimming response in terms of a single nerve net and it has been suggested that the swimming response is controlled by a separate system⁴. The electrical activity from sea anemones can be measured and correlated with various behaviour patterns^{5,6}. Use of these techniques with *S. coccinea* has shown that a slow conduction system, quite distinct from the familiar through-conducting nerve net⁷ always becomes active when *Dermasterias* contacts *Stomphia*. This activity, it seems, triggers the entire swimming sequence.

Polythene suction electrodes were used for both recording and electrical stimulation, as described elsewhere⁸. An electrode was attached to the tentacles of the anemone for recording purposes and the animal was harnessed (Fig. 1a) by pinning a sector of the oral disk of a completely intact anemone to a rigidly fixed platform of Sylgard resin. This ensured that those tentacles within the sector would be fixed in space, thereby reducing the possibility of the electrode being thrown off during swimming movement. This procedure does not unduly affect the swimming response.

A single shock (5 ms duration, 10 V intensity) delivered through a stimulating electrode attached to a tentacle evokes a response (Fig. 1b), detected by a recording electrode on an adjacent tentacle. Similar pulses have been recorded from other sea anemones^{5,9} and seem to originate from three separate conduction systems: the through-conducting nerve net (conduction velocity up to 120 cm s⁻¹)

and two slowly conducting systems, the SS1 (conduction velocity 3–14 cm s⁻¹) and SS2 (conduction velocity ~ 4 cm s⁻¹). The properties of these systems have been described elsewhere⁶. Activity occurring within any one system may be distinguished from that occurring in the others by comparing the conduction velocities, thresholds of excitation and characteristic waveforms of the recorded pulses (see Fig. 1b).

The complete swimming sequence can be triggered by bringing *Dermasterias* into contact with a single tentacle of *S. coccinea*. Previous accounts^{1,2} have described the stages in this behaviour pattern as follows: (1) an "alerting" response of the entire tentacular crown on contact with the starfish; (2) temporary contraction of the upper region of the column often shielding the tentacles from view and breaking the tentacle–starfish contact; (3) extension of the column because of a gradual constriction of the mid-column region; (4) re-emergence of the tentacular crown, relaxation of the upper column region and expansion of the entire oral disk; (5) constriction of the pedal disk margin, detachment of the pedal disk from the substrate and formation of a cone-shaped projection from its centre; (6) alternating ipsilateral and contralateral flexions of the column wall constituting the swimming movements of the animal. The entire sequence, stages (1)–(6) may take ~ 5–15 s, and the swimming flexions continue, with lengthening pauses between bursts, for up to 5 min.

The electrical activity recorded from a single tentacle of the anemone at the beginning of this response is shown in Fig. 1c. In this instance, the starfish was placed against the tip of a single tentacle, 4.5 cm from the recording site, for 1.5 s. The evoked electrical activity was a series of pulses identified by their waveforms as originating in the SS1. There was no accompanying activity associated with the through-conducting nerve net or SS2. This was not because the recording equipment could not detect ongoing activity in these systems, since a test shock applied during the response evokes characteristic pulses in all three systems. The behavioural responses accompanying this electrical activity included stages (1)–(5); but no electrical activity has yet been recorded, either from the SS1 or any other identifiable system, during the swimming flexions (stage (6)).

The SS1 can be electrically stimulated alone by placing a stimulating electrode on an ectodermal flap cut into the column and applying shocks at an intensity above SS1 threshold⁶. With the exception of the temporary, upper-column contraction (stage (2)), the entire swimming response can be triggered by stimulating the SS1 within the appropriate frequency range; about 1 shock every 250 ms to 1 shock every 4 s. At greater frequencies, the SS1 becomes refractory and does not conduct. At lower frequencies, the

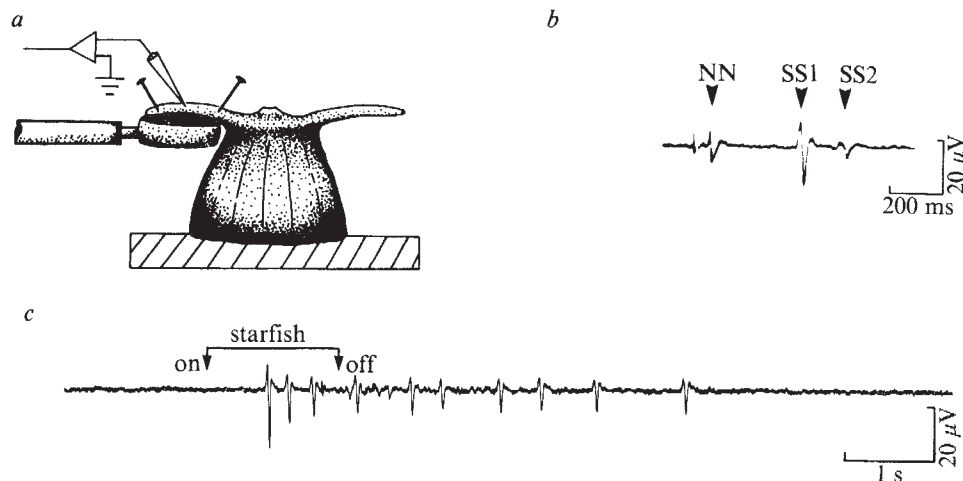


Fig. 1 *a*, *Stomphia coccinea* tethered to an immobile platform for recording purposes. A suction electrode is attached to a tentacle pinned to the platform and the pedal disk has affixed itself firmly to the substrate. *b*, Electrical activity recorded from a tentacle in response to a single shock to an adjacent tentacle. The first pulse denotes the stimulus artefact. The recorded pulses are associated with three separate conducting systems: the through-conducting nerve net (NN) and two slowly conducting systems, SS1 and SS2. *c*, Electrical activity recorded from a tentacle during the initial stages of the swimming response. The starfish *Dermasterias imbricata* was brought into contact with another tentacle to initiate the response (on) and removed 1.5 s later (off). This evokes repetitive firing in the SS1.

swimming response cannot be triggered. A minimum of about 4 shocks (at a frequency of 1 shock every 250 ms) is usually required to elicit the response but more shocks were often required if the anemone had not swum within about the preceding 10 h. This indicates that the internal state of the animal may exert an influence over the activation threshold of the swimming response¹⁰.

The through-conducting nerve net and SS2 do not seem to be involved in triggering the swimming response. Electrical stimulation of the intact column above SS1 threshold excites both the nerve net and the SS1, but in this case the swimming response may be triggered with a slight modification: the anemone sometimes contracts or 'twitches' as the shocks are applied. If the stimulus intensity is above threshold for the nerve net but below that for the SS1 so that the nerve net alone is stimulated, the anemone will contract but swimming behaviour is not triggered. This confirms and explains earlier results with electrical stimuli applied to the intact column⁴ where the threshold for swimming was shown to be higher than that for contraction. The failure of electrical stimulation of the SS1 to cause the initial contraction of the column (stage (2)) that occurs in the response to *Dermasterias* is, nevertheless, puzzling. This stage in the response may be caused by mechanoreceptors¹⁰ activating a system whose electrical activity cannot be detected by the techniques used here.

The SS1 pulses seem to constitute a sensory response to the unidentified active chemical found in *Dermasterias*¹¹. This sensory response operates over a much faster time scale than comparable activity previously encountered in sea anemones. In Fig. 1c a total of 10 pulses occurs over a period of 4.8 s (mean inter-pulse interval (i.p.i.) = 533 ms). In the response of the anemone *Calliactis parasitica* to *Buccinum* shells, the SS1 fires at a mean i.p.i. of approximately 7 s (ref. 12), and in the prefeeding response of the anemone *Tealia felina*, the same system fires at a mean i.p.i. of approximately 25 s (ref. 13). Anti-facilitation of pulse amplitude occurs in addition to a process of sensory adaptation (Fig. 1c) phenomena also encountered in *Tealia*¹³.

The SS1 coordinates pedal disk detachment in *Calliactis*¹² and it probably has a similar function in *Stomphia*, operating the entire process at a much faster rate. The observation that continuous activity of the SS1, SS2 or through-conducting nerve net is not necessary to maintain the swimming flexions suggests that the SS1 may feed into and trigger a 'swimming programme' which is maintained and coordinated by a localised motor system, perhaps the network of large multipolar nerve cells found in the column¹⁴. So far, however, it has not been possible to record electrical activity from this network during swimming.

These results extend the direct evidence for the view that the behaviour of sea anemones is not controlled by a single nerve net. Swimming in *Stomphia*, like the transfer of *Calliactis* to shells¹² and other complex behaviour patterns, involves interactions between different conduction systems. These findings should allow a reappraisal of current ideas about the control of behaviour and the organisation of the nervous system in these so called 'simple' animals. Species of *Stomphia*, which display a variety of specific, stereotyped and repeatable behaviour patterns, should be prime subjects in further investigations to the end.

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Polarised light-sensitive interneurons in a swimming crab

THIS report shows that there are polarisation-sensitive interneurons in the crab visual system, contrary to recent speculations¹. It also shows that polarised light information in crabs is elaborately processed.

Naturally-occurring light polarisers include water surfaces, particles suspended in air and water², and many larger objects, especially of biological origin (unpublished observations). Five uses of this diversity of environmental polarised light by animals have been put forward. Animals such as bees^{3,4} may infer the position of the sun for navigation although the sun itself is obscured. Polarised light patterns may enable aquatic animals to detect an 'artificial horizon'. For animals flying above water surfaces this may result from the fact that reflected light is horizontally polarised⁵. In the underwater case light is polarised by reflection from suspended particles; here the area of greatest brightness is always horizontally polarised⁶. The water skater *Gerris* may use polarisation sensitivity (PS) to screen out the predominantly single plane of reflected light from water surfaces, enabling a better view into the water⁷. PS may enable extra discriminations about visual contrast through a system analogous to colour vision. This approach has been suggested for human divers⁸, but has not until now been proposed as a physiological mechanism. Finally, PS in photoreceptors has been shown to enhance quantum capture and thus absolute sensitivity to light⁹.

In spite of this wealth of possibility, evidence of the interneuronal processing which must underlie the behaviours listed above has been presented in only one, anomalous, case: PS interneurons have been reported in the goldfish²; the anomaly is that goldfish receptors exhibit no PS.

I looked for PS interneurons in the optic lobes of the eye of a swimming crab, *Scylla serrata*, an animal which has known PS in the receptors (ref. 10 and unpublished observations) (Fig. 1a and b). I used a broad field stimulator not reported before in polarised light vision research, which in some ways simulated the light environment found underwater. This paper presents evidence of PS interneurons in *Scylla*: the result does not agree with the recent suggestion that the neural wiring pattern of the crustacean eye cancels out the PS information present in the receptors, producing only a polarisation-independent system¹.

In the experiments reported here, single spots were projected from behind on to a paper diffusing screen 30 cm from the rigidly cemented left eye of a legless but otherwise intact crab. The screen measured 16×9 cm, and on it spots from 2 to 30° diameter subtended at the eye could be displayed. All stimuli were plane polarised by a 60-cm diameter rotatable disk of polaroid positioned between the screen and the eye. A 360° potentiometer mounted coaxially with the disk enabled its position, and its rate and direction of rotation, to be recorded.

In the experiments, two essential precautions were taken. The problem of spurious reflections dependent on the light source had already been recognised¹¹. These were avoided

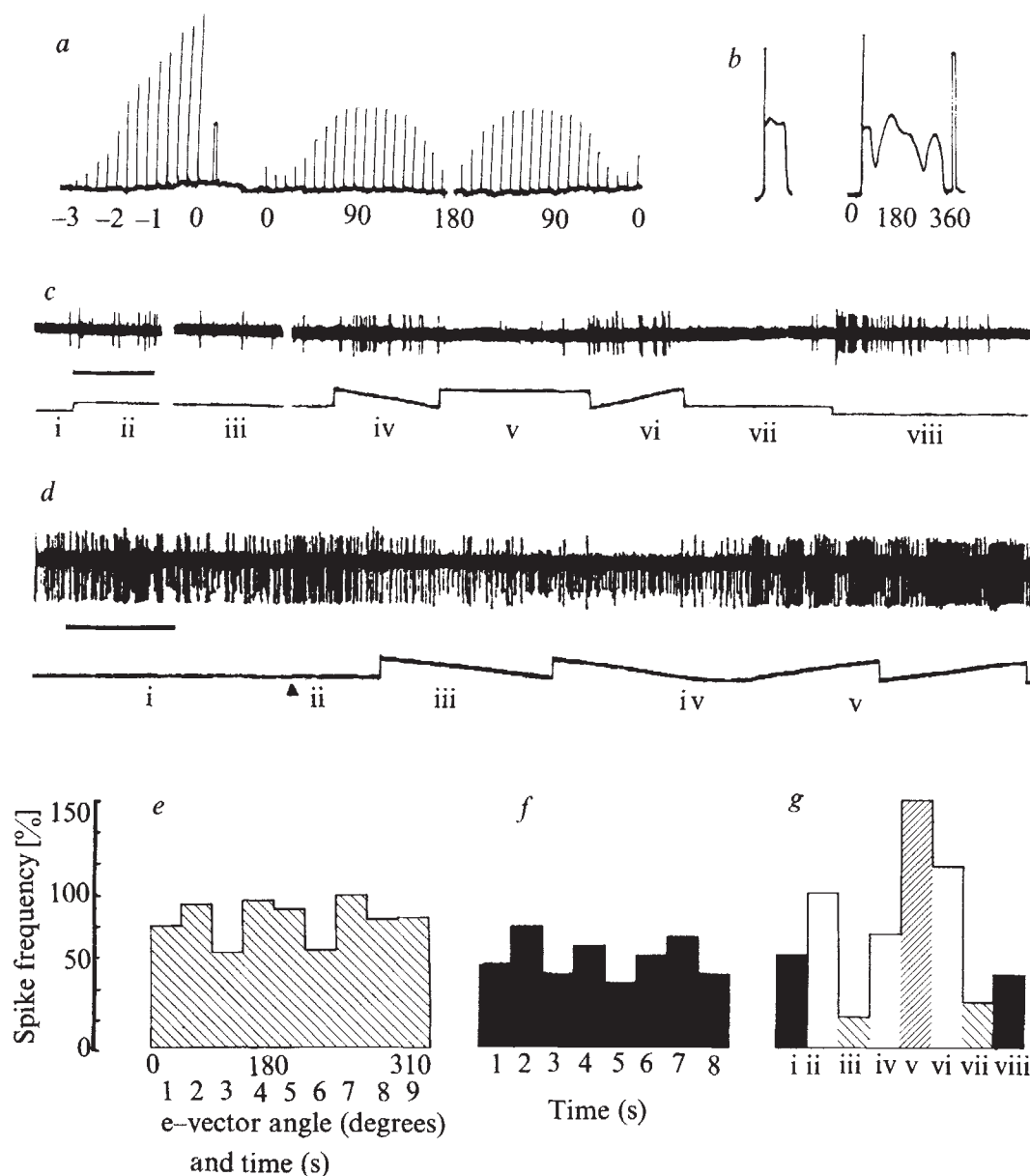


Fig. 1 *a*, Photoreceptor responses to increasingly intense flashes of light over 3 log units of intensity, and then photoreceptor responses to flashes of polarised light with the e vector moved first clockwise and then anticlockwise in 10° steps, one 40-ms flash every 5 s. Calibration 20 mV. The difference in response at maximum and minimum is equivalent to 1 log unit of intensity and thus the cell has a polarisation sensitivity of 10. *b*, Photoreceptor responses to continuous polarised light, e vector stationary and vertical, and then to continuous polarised light, e vector rotated through 360°. *c*, Interneurone I. The stimulus channel shows: i, dark discharge; ii, light-on (polaroid static and vertical); iii, response after 30 s of this unchanged stimulus; iv, polaroid rotation clockwise 360°; v, polaroid left, stationary and vertical; vi, polaroid rotation anticlockwise; vii, polaroid stationary and vertical; viii, light-off. Time mark 5 s. Note that the response is independent of stimulus direction, and that light-off has the strongest effect. *d*, Interneurone II. The stimulus channel shows: i, dark discharge; ii, dim light-on (▲) with polaroid static and vertical; iii, polaroid rotation clockwise; iv, polaroid static and vertical; v, polaroid rotation anticlockwise. Time mark 5 s. Note directional selectivity of response, and that polaroid rotation has a stronger effect than light on. *e-g*, Interneurone II. Data showing absence of stationary, presence of rotational PS. *e*, Response of a unit to a stimulus similar to that given the receptor cells in continuous light but 45° steps. There is no significant stationary PS as the response range is not significantly different from a similar period of dark discharge (*f*). *g*, Distinct response of three class II units to the rotational stimulus sequence (i-viii) listed above.

by placing a black screen with a small (3×2 mm) window close (2 mm) to the eye. The screen acted as a light baffle and ensured that the crab saw only the light spot and not, for example, its reflection from the floor of the preparation holder. Second, the fact that the stimulating variable really was polarised light and not intensity was checked. Measurements with a Hewlett Packard 8334A radiant flux meter showed only 0.57% intensity variation during an e-vector rotation of 360°.

Units were recorded extracellularly from the external medulla by conventional glass microelectrode techniques. Examples of two classes of unit are presented here. Both

classes have very broad receptive fields, responding to test spots applied anywhere over the whole eye. For both units the experimental protocol was similar.

Unit 1 (20 cells recorded) responds to polaroid rotation, but is insensitive to the direction of rotation. In a typical trial (Fig. 1c, i-viii), a class I unit showed a small increase in discharge to light-on (e vector stationary and vertical). The cell was allowed to adapt to a low frequency of discharge (iii) and 30 s after light-on, polaroid rotation at a rate of about 50° s⁻¹ was commenced (iv). This led to a fivefold increase in discharge rate. The opposite direction of rotation (vi) showed the same effect, and that it was

independent of the orientation or direction of rotation of the *e* vector. Stopping rotation led to cessation, then a gradual return of activity.

Figure 1*d* and *e* show the responses of a class II unit which responds to both rotation and direction of rotation of *e* vector (6 cells recorded). Here, illuminating the spot with the *e* vector stationary and vertical, led to a doubling of discharge rate (ii). Rotating the polaroid clockwise at about 50° s⁻¹ (iii) led to a progressive decrease in rate to a fifth of that induced by light-on. In complete contrast, an anticlockwise rotation (v) led to a progressive and sevenfold increase on the previous clockwise rate.

In discussing these results a point first arises as to categories of polarised light stimulus. When a polarising filter is rotated between a light source and the crab eye, the intracellular response of a retinula cell varies with the angular position of the filter (Fig. 1*a*). The commonly used definition of receptor PS has always been derived from responses to flashes of light, each being through a different stationary *e*-vector angle. I term this stimulus a stationary stimulus. It contrasts with the stimulus used in the interneurone experiments reported here, which I term a rotational stimulus.

The receptors of the crab eye are modulated by both stationary and rotational polarised stimuli (Fig. 1*a* and *b*). Because they respond to both, receptors by themselves cannot distinguish between stationary and rotational stimuli. It is clear that the interneurons demonstrated here are more specialised elements in a polarisation detection system. Both are more highly tuned to *e*-vector rotation than the receptors; furthermore, unit II is more highly tuned to rotation than unit I.

Although there are no data on the static PS of unit I, its response to rotation is quite different from that of a receptor. It has a flat response to rotation, whereas the receptor response is modulated (Fig. 1*b*).

In turn, unit II is more highly organised than unit I. First, in terms of the weighting given to intensity change relative to *e*-vector change: in unit I, the greatest response modulation was caused by light-off; in unit II, the greatest modulation was caused by *e*-vector change. Second, and most important, only unit II responds with directional selectivity.

These results demonstrate the existence of polarised light information in the crab central nervous system. The crab may use this information in three of the five ways mentioned in the introduction.

The first of these is contrast enhancement. The units reported here have been shown to respond to changes both in light intensity and in rate of *e*-vector rotation. It may be, however, that the general response is to moving patterns composed of variations in intensity and of polarisation. If this is the case visual contrast in the system could be enhanced in the following way. It is possible to imagine a grey moving object invisible against an equally grey background. If, however, the light coming from the object were different from the background in amount and *e*-vector angle of polarised light—a situation common in the ocean—the above PS system would detect a contrast difference. In this way the otherwise invisible object would become visible.

Second, orientation: in servo systems it is advantageous that rate of change detectors feed back to the output. If information from the units reported here were fed back to the motor system, *Scylla* could swim so as to minimise output from the PS interneurons and therefore move at a fixed angle relative to the polarised background. Such a system would minimise rolling and would act as a visual stabilisation system. Note, however, that units of class I and II alone would not enable a crab to orientate to one particular angle as opposed to another. This is because neither unit measures stationary *e*-vector angle. For this, a channel carrying positional information is required. This

could be from the gravity detection system—in crabs, the statocysts—or from as yet undiscovered interneurons with stationary PS. Bodies of water are often turbid to the extent that swimming animals can see neither bottom nor solar position. In these very conditions, however, the polarised artificial horizon would be easily visible to an animal with the polarisation-sensitive system I have outlined. Such a system would enable animals to orientate visually in otherwise featureless water.

Finally, the underwater polarised pattern, while maintaining its horizontal component, moves with the Sun's movement during a day. If PS interneurons were allied with an internal clock, the system, as in the bee, could be used for navigation.

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Aerial gas exchange in Australian arid-zone crab, *Parathelphusa transversa* Von Martens

Parathelphusa transversa is a brachyuran crab of the family Potamonidae and is found over much of inland Australia, including arid areas¹. Permanent water sources, such as water holes, rivers and dams are avoided and during prolonged dry periods the animals lead a fossorial existence, their air-filled burrows being found in the clay soils of dry swamps and water courses. After heavy rain or flooding they emerge from their burrows to forage in the surface water, leading an amphibious existence. Their mode of life requires the capacity for gas exchange in both air and water and this is associated with structural adaptations of the branchial chamber to form a lung. We describe here the animal's unusual and hitherto undescribed mechanism of tidal ventilation of the lung.

Several other families of the Decapoda include terrestrial and semi-terrestrial representatives². Most attention has been paid to those crabs which evolved from intertidal forms so that it is of interest to examine the parallel adaptations to air breathing which have occurred in this crab of freshwater origin.

The branchial chambers which are enclosed by lateral extensions of the carapace (the branchiostegites) are much expanded compared with aquatic forms (Fig. 1). There are seven pair of gills (a smaller number than in most aquatic crabs). These are reduced in size and only occupy a small proportion of the total volume of the branchial chambers. The remaining epibranchial spaces are normally air-filled, when the crab has access to air.

The branchiostegite is highly vascularised as in other terrestrial crabs (Figs 1–3). Blood channels between the inner and outer integument of the branchiostegites are separated by a system of connective tissue partitions, which

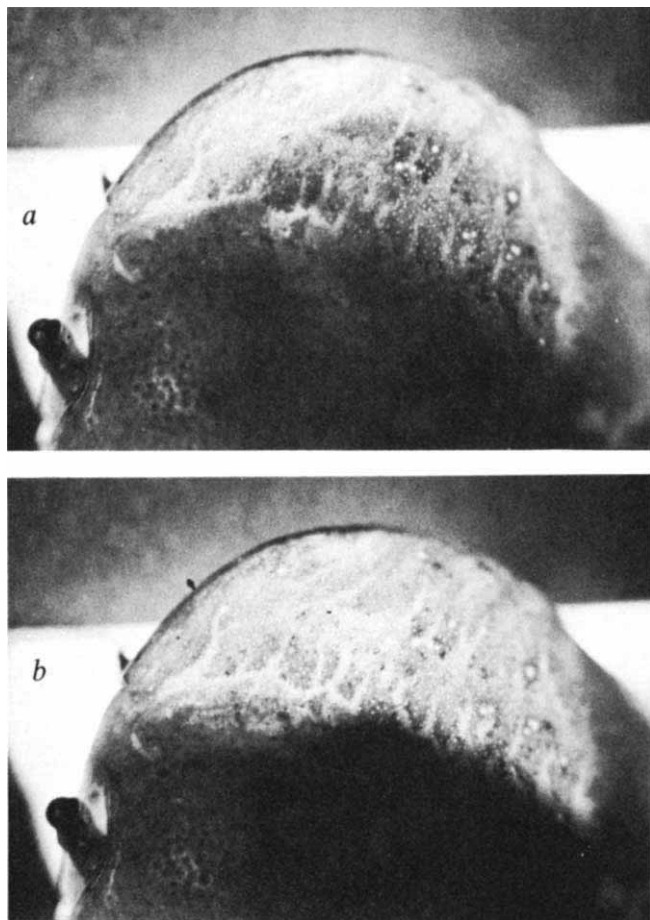
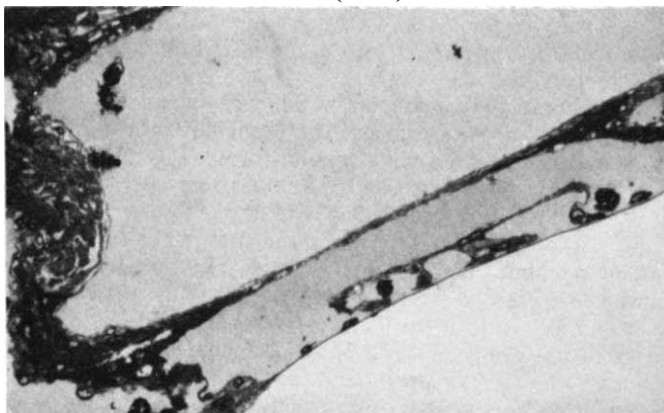


Fig. 1 *P. transversa* supported in air and illuminated strongly from the ventral side. Markings on carapace above branchial chamber delineate the major blood channels. *a*, Near-maximal expiration. Note the shadow of the thoracic wall penetrating into the branchial chamber. *b*, Maximal inspiration; thoracic wall has retracted out of the branchial chamber.

fulfil the dual role of directing blood flow and acting as a suspensory system for the inner integument. Support for this delicate membrane is presumably also provided by the hydrostatic pressure of the haemolymph.

Although the inner surface of the branchiostegite is smooth and lacks the surface amplification described in the Ocypodidae² and Coenobitidae³ it is well adapted for air-blood gas exchange. The inner cuticle (Fig. 3) is extremely thin (about 0.2 μm). For comparison, the cuticle covering

Fig. 2 0.5- μm epoxy section of dorsal region of branchiostegite stained with methylene blue. Extensive blood channels are partitioned by connective tissue. The thin epidermal lining of the inner cuticle (lower area) is unresolvable at this magnification ($\times 168$).



the gill leaflets and filaments in this and other crustaceans is generally about 1–3 μm thick (refs 4 and 5, and unpublished observations). The cuticle here seems to be represented only by a thin epicuticle⁶. There are no lamellae of helicoidally oriented chitin-protein microfibrils as seen in the exocuticle and endocuticle of the gills and other regions (ref. 6 and unpublished observations).

The blood-air diffusion path is further minimised by extreme attenuation of the epidermal cells underlying the inner cuticle. Except in the sparsely distributed perinuclear regions of the cells the cuticle is separated from the blood by a cellular layer only 20–100 nm thick, of which about 15 nm is represented by the apical and basal plasma membranes. The apical plasma membrane is applied directly to the cuticle. A thin (about 40 nm) basal lamina covers the haemolymph surface of the cells. Thus the total blood-gas distance is about 0.2 μm over most of the surface of the lung. This is of the same order of magnitude as the blood-gas distance in the lungs of the most active vertebrates and considerably less than in some⁷.

The mechanism of ventilation when breathing air has

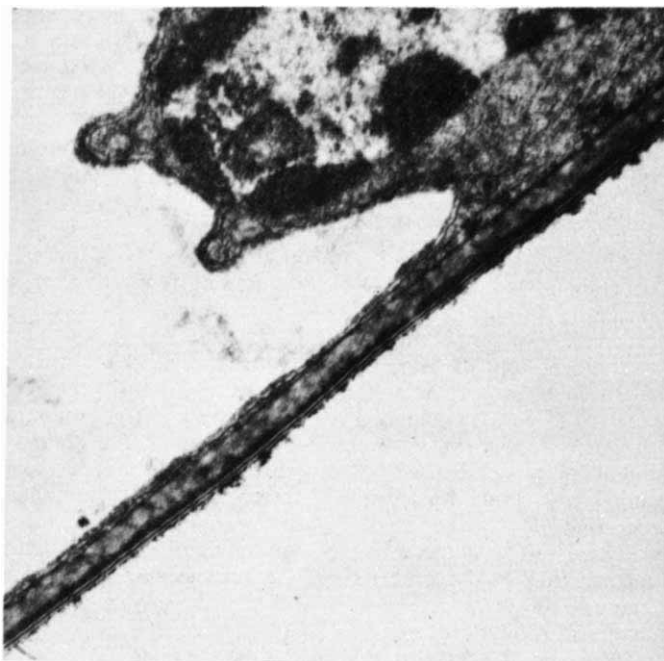


Fig. 3 Electron micrograph of inner cuticle of branchiostegite showing very attenuated extensions of the epidermal cell over the blood side of the cuticle. Formaldehyde-glutaraldehyde fixation with post-osmication ($\times 10,500$).

been examined. Surprisingly the scaphognathites, which are important in producing respiratory water currents in the aquatic mode and which are involved in aerial ventilation in other crabs^{2,3,8}, are not involved. Expiration is brought about by expansion of the membranous thoracic wall and roof of the branchial chamber (dorsal region of the thoracic epimera and pleura) into the epibranchial chamber (Fig. 1*a*). Inspiration is achieved by the retraction of the membrane to assume its normal concave form (Fig. 1*b*). Inspiration in one epibranchial chamber is accompanied by simultaneous expiration in the other. These movements may be observed by shining an intense light (with heat filter) through the animal from the ventral surface and observing the shadows of the thoracic wall on the carapace. This movement involves a slow lateral oscillation of the thoracic viscera (dark areas in Fig. 1*a* and *b* are probably mainly the digestive gland).

Direct observation reveals that the scaphognathites do not

beat during aerial ventilation, the prebranchial apertures above the mouth remaining open. By placing detergent films over these so-called 'exhalant apertures', it was established that ventilation was tidal, both inspiration and expiration occurring through these apertures. The Milne-Edwards apertures at the base of the chelipeds are not involved in aerial respiration. In another freshwater crab, *Trichodactylus petropolitanus* (Trichodactylidae) the scaphognathites cease beating in air, although it was considered that air entered the branchial chamber by diffusion during aerial respiration in this animal⁹.

In resting *Parathelphusa* the ventilatory movements are sporadic but after exercise become regular in frequency and depth, reaching a maximum rate of 20 cycles min⁻¹ (each cycle represents one inspiration and expiration for each lung). During maximal ventilation the thoracic wall moves almost completely across the epibranchial chambers. It is estimated that at least 70–80% of the air in the lung is exchanged in each ventilatory cycle.

The oxygen extraction efficiency of the lung of *Parathelphusa* has not yet been measured. Ventilation of the gas exchange surface seems, however, to be more efficient than in *Birgus* or gecarcinids, where diffusion distances between the branchial tufts or gills are relatively long⁴.

The muscular basis of ventilation is not yet known. It is possible that muscles homologous to the 'dorso-ventral muscles' described by Pearson¹⁰ in *Cancer* are involved. These muscles run from the roof of the branchial chamber to the carapace. Contraction of these could effect inspiration while expiration in the contralateral branchial chamber would be simultaneously effected by hydraulic displacement of the haemolymph and viscera.

No similar ventilatory movements have been described in other crabs, although in *Birgus* carapace movements contribute in a minor way to air flow through the lungs⁵. Unlike a vertebrate, a crab's total volume is essentially fixed by its rigid exoskeleton. In this light the simultaneous inspiration and expiration in opposite chambers could be seen as a neat solution to the problem of lung ventilation.

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of the test medium, $[Ca]_0$, is raised above 10^{-6} M (ref. 1). Similarly, extracted cells of the typanosomid *Crithidia* show a reversal in wave propagation that is controlled by Ca ions. In studies on the intact flagellar apparatus isolated from a wall-less mutant of *Chlamydomonas reinhardtii*, the flagella have been shown to reverse direction of the effective stroke when external $[Ca]_0$ is raised above 10^{-6} M (ref. 3). Electrophysiological evidence indicates that, in *Paramecium*, reversal of ciliary beating is caused by Ca which enters the cell when the Ca permeability of the membrane is increased^{4,5}. We have investigated whether Ca is involved in the phototactic response and more specifically whether this Ca influx is regulated by the cell membrane. Electrophysiological recording was impractical because of the small size of these cells ($< 10 \mu\text{m}$), and so the approach was to photograph the light-induced motor responses of cells swimming in solutions containing different concentrations of Ca or Ca-blocking agents. The results demonstrate that the reversed beating response of the flagella depends on Ca. Inhibition of the response by Ca-blocking agents supports the view that reversed beating response of the flagella is coupled to photostimulation by an influx of Ca ions.

Wall-less mutants of *C. reinhardtii* strain CW92 were grown axenically in liquid medium⁶ at 25 °C with constant shaking under a 14-h light, 10-h dark cycle. Light was provided by Cool-white and Gro-lux fluorescent illumination at an intensity of 2.6 W m^{-2} . Response variability was minimal when cells were used between hours 67 and 77 after inoculation and between hours 3 and 6 of the light cycle.

Cells were centrifuged and resuspended twice in the experimental solution and were kept in the dark before transfer to a microscope slide. They were then examined by phase illumination under red light (Corning glass 2-62 sharp-cut filter, 600–750 nm) and left undisturbed while exposed continuously to the red light for 60–75 min before stimulation. Red illumination did not produce any overt behavioural response in these cells. The stimulus, a substitution of white light for the red light, was achieved by electromechanically replacing the red filter by a neutral density filter for approximately 280 ms. The red light had an energy before entering the microscope optics of $\sim 80 \text{ W m}^{-2}$ and the white stimulus light plus neutral density filter $\sim 70 \text{ W m}^{-2}$. The temperature at the microscope stage was 20 ± 1 °C. A fresh sample of cells was used for each experiment and each group of cells was stimulated only once.

The control solution contained 2 mM EGTA (ethylene glycol-bis-(β -aminoethylether)-*N,N'*-tetraacetic acid), 3 mM CaCl_2 , 1 mM KCl, 0.1 mM MgCl_2 , and 5 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid) at pH 7.1. Experimental solutions were identical except for the concentration of Ca or the addition of test substances as described below. CaCl_2 was added to the fixed concentration of EGTA in amounts yielding the desired concentration of free Ca for $[Ca]_0$ between 10^{-8} and 10^{-5} M (ref. 7). At higher concentrations the free Ca was regarded as the total Ca concentration minus the EGTA concentration.

Analysis of cell and flagellar motion was carried out at $1,000\times$ image magnification on Kodak 2496RAR film exposed in a LOCAM 16-mm camera at 200 frames per s. Frame-by-frame analysis of the recorded sequence was made on a Vanguard motion analyser, and representative flagellar configurations were traced by hand, and reproduced schematically in Fig. 1a. Backward swimming velocity is defined as the distance covered by the cell during an episode of backward swimming divided by the time elapsed during reversed locomotion. Forward swimming velocity was calculated from the distance covered by the cell in 150 ms. In those instances where forward locomotion was determined during a light stimulus the period of measurement began 20 ms after stimulus onset.

During forward swimming under red light the flagellar motion resembled a breaststroke^{8,9} (Fig. 1A, bottom left, a). The power stroke consisted of the simultaneous movement of both flagella in a rather straight sweep bending only near the

Calcium couples flagellar reversal to photostimulation in *Chlamydomonas reinhardtii*

SEVERAL recent findings^{1–3} suggest that the intracellular concentration of free calcium, $[Ca]_i$, has an important role in the regulation of ciliary and flagellar beating. For example, *Paramecium* extracted with Triton X-100 and reactivated with Mg^{2+} and ATP swim backwards when the Ca^{2+} concentration

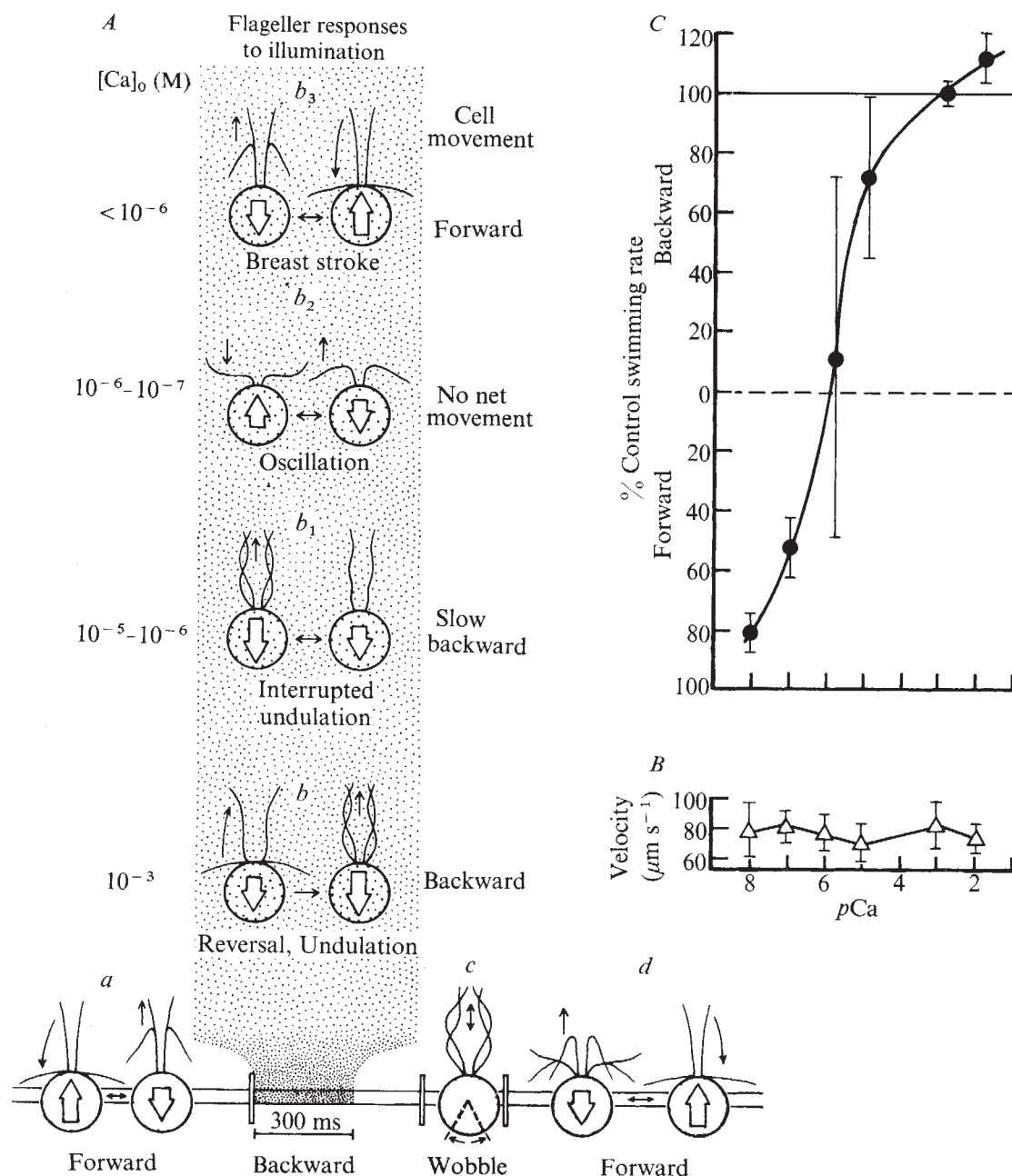
base. This stroke propelled the cell forward with the base of the flagella leading. The recovery stroke consisted of a wave propagating along the flagella from base to tip, which pushed the cell backwards slightly. A complete cycle of beating lasted about 20 ms. The time required for one complete cycle was determined to ± 10 ms by measuring the time required for 5 beats to ± 10 ms.

Photostimulation in the control solution always produced a constant modification of flagellar movement and hence swimming behaviour (Fig. 1). The cell continued to swim forward for about one cycle (that is, 20 ms) after the onset of the stimulus and then stopped for about 20 ms. The power stroke

then reversed (reversal in *b*, Fig. 1*A*), increased in frequency and caused the cell to back up. This reversed swimming was only seen in response to the photostimulation. Backward swimming continued for 200 ms after the stimulus. After 550 ± 100 ms from the initiation of backward swimming the cell eventually slowed to a stop and for about 200 ms wobbled around its transverse axis (*c*, Fig. 1*A*). The cell moved about $14 \mu\text{m}$ in the reverse direction before resuming forward movement.

Changes in $[\text{Ca}]_0$ did not significantly affect forward swimming in the unstimulated cell (Fig. 1*B*) but markedly modified the light-induced responses (Fig. 1*A*, b_1 – b_3). In the range of

Fig. 1 Dependence of *Chlamydomonas* photoresponse on $[\text{Ca}]_0$. *A*, Bottom, normal sequence (*a*, *b*, *c* and *d*) of flagellar behaviour and cell movement before, during and after a 300-ms light stimulus in control (that is 10^{-3} M Ca) solution. Durations of backward swimming and wobble are about 550 and 200 ms respectively. Reversal and termination of backward swimming latencies are 20 and 200 ms respectively. The response during light is shown on a larger scale in the expanded portion of the stippled area. Backward locomotion was progressively inhibited (b_1 , b_2 , b_3) by reduced $[\text{Ca}]_0$. *B*, Prestimulus velocity ($\mu\text{m s}^{-1}$) plotted against $p\text{Ca}_0$. Bars give s.e.m. There was no significant difference in forward swimming velocity at $[\text{Ca}]_0$ between 10^{-8} and 10^{-2} M. *C*, Rate of swimming during photostimulation plotted against $p\text{Ca}$ as percentage of prestimulus control swimming rate. Control swimming rates for both forward and backward locomotion represent rates measured at 10^{-3} M $[\text{Ca}]_0$. Backward locomotion was normalised to the forward control swimming rate. Bars give s.e.m. Backward locomotion is plotted above, and forward locomotion below the dashed line. Control swimming rate was restored after cells were transferred from a 10^{-8} M Ca solution to the control solution, 10^{-3} M.



10^{-5} to 10^{-6} M Ca there appeared interrupted undulations of the flagella (b_1 , Fig. 1A). In addition, the velocity of reversed swimming became progressively slowed. At 10^{-6} M Ca the flagella of about 10% of the cells exhibited an oscillation in the focus plane which produced no net cell movement (b_2 , Fig. 1A). At concentrations less than 10^{-6} M Ca no reversed beating was observed in response to the stimulus (b_3 , Fig. 1A) but there was a significant slowing of forward motion.

The rate of swimming in response to photostimulation is plotted against pCa in Fig. 1C. The highest rate of backward swimming recorded was in 10^{-2} M Ca solution. At 10^{-6} M some cells swam backwards slowly while others swam forward. At less than 10^{-6} M backward swimming could not be evoked with photostimulation; swimming continued in the forward direction during photostimulation with the highest observed rate at the lowest Ca concentration, 10^{-8} M. The effects of reduced Ca concentration on the response to photostimulation were reversed completely when cells were returned to the control solution.

The velocity of light-induced backward swimming was measured in the presence of agents known to block Ca influx in excitable membranes. Addition of 10^{-2} M Ba or 10^{-2} M Mg to the control solution resulted in a reduced velocity of backward swimming (Fig. 2). The effect was fully reversible. In addition, the organic Ca-blocking compound D-600 (α -isopropyl- α -[(N-methyl-N-homoveratryl)- γ -aminopropyl]-3,4,5-trimethoxyphenylacetone nitrile)¹⁰ inhibited backward swimming at 10^{-4} M or greater (Fig. 2). In contrast to Ba and Mg, however, the inhibition caused by this drug was irreversible. Procaine, a local anaesthetic which reversibly blocks membrane excitability¹¹ and is reported to be a competitor of Ca in the

lobster axon¹², was found to block reversibly backward swimming at 10^{-2} M or greater (Fig. 2).

Lanthanum, Ni, Co and Mn ions, which generally block Ca currents in other membranes¹³, have no effect on backward swimming at concentrations up to 1, 5, 50 and 80 mM respectively. At higher concentrations these cations were toxic. These agents also fail to block in *Paramecium* the Ca influx which mediates ciliary reversal^{4,5,14,15}.

The results presented here indicate that photostimulated flagellar reversal depends on extracellular Ca. The inhibition of the response by certain Ca-blocking agents such as D-600, procaine, Ba, and Mg, which are known to block Ca influx in other systems, suggests that photostimulation normally results in an influx of Ca through the cell membrane, producing a transient increase in $[Ca]_i$. The induction of reversed beating by Ca in the isolated basal body-flagellar apparatus³ of *Chlamydomonas* supports this conclusion. Thus it seems that Ca^{2+} acts as the agent that couples flagellar responses to photostimulation in flagellate. This is reminiscent of the role of calcium in coupling ciliary reversal to membrane stimulation in the ciliated protozoa.

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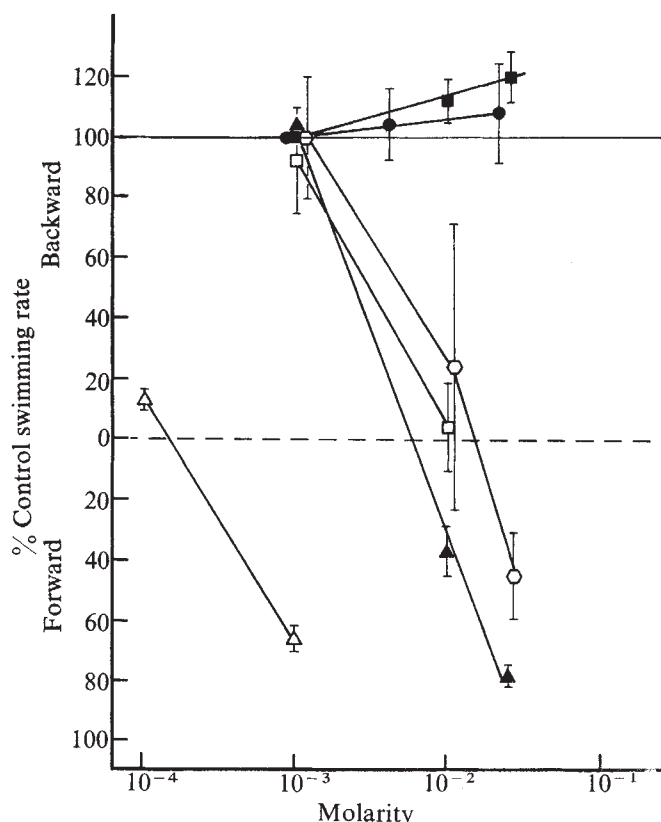


Fig. 2 Effect of several agents on backward swimming velocity in the presence of 10^{-3} M Ca. Percentage of swimming rate in control solution is plotted against concentration of each agent. Bars give s.e.m. Procaine concentrations were maintained at a constant 20 mosM with pentaerythritol. Ba, Mg and procaine inhibition was fully reversed by return to control solution. ●, Pentaerythritol; ▲, procaine; △, D-600; □, Ba^{2+} ; ■, Ca^{2+} ; hexagon, Mg^{2+} .

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Bioconversion of light energy into chemical energy through reduction with water of nitrate to ammonia

PHOTOSYNTHESIS is first and foremost a process for converting the radiant energy of sunlight into physiological chemical energy, that is, reducing power and high energy phosphate bonds¹. Since, for historical and quantitative reasons, however, photosynthesis in a broad sense is commonly identified with the reduction of carbon dioxide to carbohydrate, the fact is often overlooked that photosynthetic organisms manufacture their most representative biomolecules—proteins and nucleic acids—not only from carbon dioxide, but also from other oxidised inorganic nutrients, especially nitrate, that, more or less directly, has to be reduced to ammonia by water before being incorporated into amino acids^{2,3}. It was first thought⁴ that the reduction of nitrate in green cells was a process far removed from the light reactions of photosynthesis and that carbohydrates were the *in vivo* source of reducing power for nitrate reduction, but it now seems that, at least in blue-green algae⁵⁻⁷, the photosynthetic reduction of nitrate is even more direct than that of carbon dioxide, since it does not depend on adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH), but only on reduced

Table 1 Photoreduction of nitrate to nitrite and ammonia coupled to oxygen evolution by particles of *Anacystis nidulans*

System	Nitrite formed (μmol)	Ammonia formed (μmol)	Oxygen evolved (μmol)
Complete	0.9	2.0	4.1
-Fd	0.7	1.6	3.5
-NO ₃ ⁻	0	0	0
+CMU	0	0.2	-0.4
Dark	0	0.2	-0.5

The complete system included in a final volume of 3 ml: 150 μmol Tricine-KOH buffer, pH 8.0; 30 μmol MgCl₂; 20 μmol KNO₃; 40 nmol *A. nidulans* ferredoxin (Fd); *A. nidulans* particles containing 0.63 mg chlorophyll. Where indicated, 0.3 μmol of 3-(*p*-chlorophenyl)-1-1-dimethylurea (CMU) was added. The reaction was carried out in air at 30 °C in Warburg vessels illuminated from below (20,000 lx) or kept in the dark. Reaction time 90 min. Other analytical methods as previously described⁶. Particles were prepared as follows: cells grown on nitrate in the light, as described previously⁶, were collected in the exponential phase of growth, washed with 50 mM Tricine-KOH buffer, pH 8.0, 0.5 M sucrose, 10 mM MgCl₂, resuspended in the same buffer and broken with a precooled French press at 16,000–18,000 pound inch⁻². After digestion of the broken material with DNase and RNase for 10 min at 2 °C, the suspension was centrifuged at 3,000*g* for 10 min, the pellet discarded, and the supernatant centrifuged again at 40,000*g* for 20 min. The resulting sediment was washed once with the buffer, collected after centrifugation at 40,000*g* for 30 min, and finally resuspended in the buffer. to be used as a preparation of particles.

ferredoxin, the first electron acceptor from photosystem I. Also the enzymes for the reduction of nitrate to ammonia are retained tightly bound by the pigment-containing particles from these prokaryotes.

We report here the preparation of active and stable particles from the blue-green alga *Anacystis nidulans*, which, in the light and in aerobic conditions, can use water as the electron donor for the steady reduction of nitrate to nitrite and ammonia without the requirement of any exogenous enzyme system (Table 1).

During the past 15 yr, the assimilatory nitrate-reducing system, its regulation and its relation to photosynthesis have been studied, using a great variety of organisms^{3,8,9}. Our results, as well as those from other groups¹⁰, have firmly established that the nitrate-reducing enzyme system consists of only two metalloproteins—nitrate reductase and nitrite reductase—which catalyse in series the reduction of nitrate to nitrite and ammonia, through an unexpectedly short sequence of reactions



Both enzymes are ferredoxin-dependent in the photosynthetic prokaryotes, but only nitrite reductase has such a dependence in the photosynthetic eukaryotes, a group which includes algae and higher plants. Nitrate reductase from eukaryotes is an NAD(P)H-dependent enzyme complex.

We have previously obtained isolated pigment-containing particles from *A. nidulans* that could carry out the ferredoxin-dependent photosynthetic reduction of nitrate and nitrite with the couple ascorbate-dichlorophenol indophenol as an artificial electron donor for photosystem I. The coupling of the process with the photolysis of water remained, however, an unsolved problem. There is also at the present time a direct interest in the photolysis of water by photosynthetic systems for fuel production, especially hydrogen gas, but these systems require auxiliary enzymes from other sources, and the removal of even traces of molecular oxygen is an absolute requisite¹¹. Biological photofixation of nitrogen is also an anaerobic process but with high ATP requirements and its coupling with water photolysis will be even more difficult to accomplish (compare ref. 12).

In the system described here the electrons derived from the photolysis of water at photosystem II are transported to photosystem I and used for the stepwise reduction of nitrate to

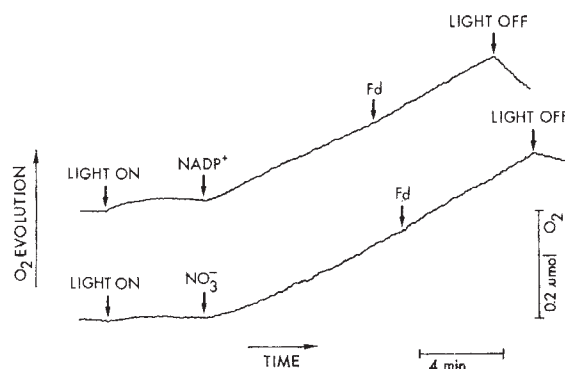
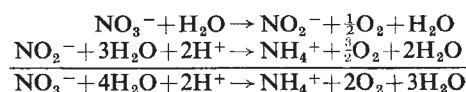


Fig. 1 Time course of oxygen evolution by *Anacystis nidulans* particles with either NADP⁺ or NO₃⁻ as 'natural' Hill reagents. The reaction was carried out at 30 °C in the chamber of an oxygen electrode, either in the light (40,000 lx) or in the dark as indicated. Other experimental conditions were the same as in Table 1, except that a particulate preparation equivalent to 0.31 mg chlorophyll was used and that 4 μmol of NADP⁺ were added as indicated in the corresponding system.

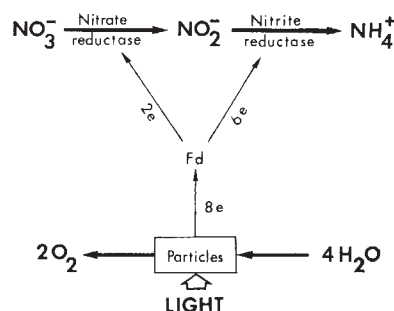
ammonia by the ferredoxin-dependent nitrate-reducing system, oxygen being evolved in stoichiometric amounts, according to the equations



It can be seen in the table that the reaction depended on nitrate, was slightly stimulated by ferredoxin, and did not proceed in the dark or when an inhibitor of the photolysis of water was added. In the conditions of the experiment, the activity of the system was linear for at least 90 min. The small O₂ uptake in the presence of the inhibitor or in the dark might be related to the presence of an endogenous reducing material in the preparation.

Gerhardt and Trebst¹³ have reported the ferredoxin-dependent non-cyclic photophosphorylation coupled to NADP⁺ reduction and oxygen evolution by *A. nidulans* preparations obtained by vacuum drying. NADP⁺ photoreduction by ferredoxin-NADP reductase and nitrate photoreduction by the ferredoxin-nitrate-reducing system are thus similar reactions. As Fig. 1 shows, our *A. nidulans* particles exhibited the same photosynthetic activities with either NADP⁺ or nitrate as 'natural' Hill reagents¹⁴. Particles stored for 1 d at 2 °C remained fully active with respect to its nitrate-dependent, oxygen-evolving capacity, except for the ferredoxin requirement, that stimulated activity twofold after several hours and threefold after 24 h. Cell-free preparations from another blue-green alga—the N₂-fixer *Nostoc muscorum*—behave in a similar way (unpublished work from this laboratory).

Fig. 2 Diagrammatic representation of the ferredoxin-dependent photosynthetic reduction of nitrate to ammonia with water as the electron donor by blue-green algae particles. Fd, Ferredoxin.



In summary, subcellular preparations of blue-green algae can utilise the photochemically-generated reducing power coming from water for the direct reduction of nitrate to ammonia. The process can be considered as one of the most simple and relevant examples of photosynthesis. According to present knowledge, the ferredoxin-dependent photosynthetic reduction of nitrate and nitrite is shown in Fig. 2. Since 16 quanta of light are required to transfer 8 electrons from water to completely reduce one nitrate, the efficiency of the process (disregarding coupled photophosphorylation) is about 13%. Besides its biochemical and chemical interest, ammonia is an excellent fuel and can also be regarded as a portable source of hydrogen¹⁵.

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Cell differentiation without morphogenesis in *Dictyostelium discoideum*

To understand pattern formation in an organism one needs to know what factors control the differentiation of each cell type and what elements are responsible for the spatial arrangement of these cell types. In the cellular slime mould *Dictyostelium discoideum*, the problem is relatively simple since the mature fruiting body consists of two basic cell types—stalk cells and spores. The stalk cells of the mature fruiting body are derived from the anterior cells of the multicellular masses formed by aggregation, whereas the spores are derived from the posterior cells¹. Cells that are plated at a density too low for aggregation, or are allowed to aggregate under water do not normally differentiate into stalk or spore cells. In many of our experiments we have made use of the fact that cells plated on agar at any density fail to undergo development beyond the stage of aggregation if they are covered by a thin layer of Cellophane. In this paper we present observations on cell differentiation which derive from the original report of Bonner² of stalk-cell induction by cyclic AMP in isolated cells. We have found that stalk-cell induction by cyclic AMP is markedly dependent on cell density, and present evidence for the involvement of a low molecular weight diffusible factor in this process. We also describe the isolation of a mutant which gives rise to spore cells under cellophane.

Bonner² reported that a proportion of amoebae of strain NC4 developed into large vacuolated stalk-like cells when plated on agar containing 1 mM cyclic AMP at cell densities

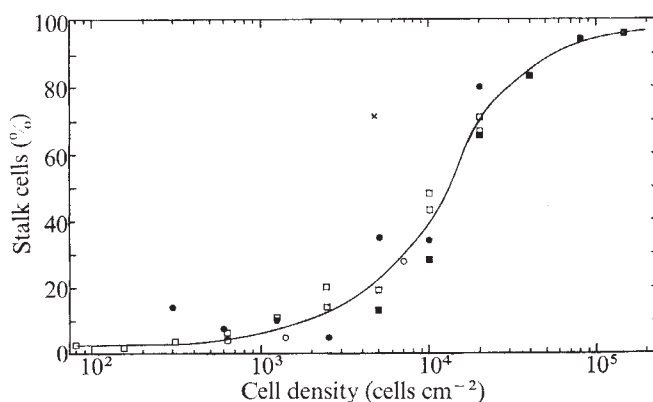


Fig. 1 Effect of cell density on stalk-cell induction by cyclic AMP. Cells were grown for 18–24 h with *K. aerogenes* on SM agar at 22 °C. They were collected and washed three times in 1% Bonner's salts and then spread at various cell densities on non-nutrient stalk-cell agar plates with or without cyclic AMP. The stalk-cell plates contained 1% Difco Bacto agar in 1% Bonner's salts, 100 µg ml⁻¹ streptomycin sulphate, 25 µg ml⁻¹ Calcofluor White 'New M2R' (Cyanamid, UK) with or without cyclic AMP; a total volume of 2.0 ml in a 5-cm plastic Petri dish. The plates were incubated in moist, dark conditions for 2–3 d and then examined by phase and fluorescence microscopy. Independent experiments are shown by different symbols. Cyclic AMP concentration was 1 mM in all experiments except one at the highest cell densities (■) when it was increased to 5 mM to prevent fruiting and ensure efficient-stalk cell induction. All control plates had less than one stalk-cell in 200 cells examined.

that were too low to allow aggregation in controls without cyclic AMP. This observation was extended by Chia³ who showed that when cells of P4 (a 'stalky' mutant of strain NC4) were plated at high density on a Cellophane support over a reservoir of 1 mM cyclic AMP, they collected into mounds in which all the cells differentiated into stalk cells. Cells of the parent strain NC4 collected into similar mounds, but only a proportion of them differentiated into stalk cells. In conditions similar to those of Bonner² (described in Fig. 1) we found that strains NC4, NC4/P4 and NC4/Ax-2 differentiate at a relatively low frequency, whereas strain V-12 M2 formed almost 100% stalk cells. Thus when cells were spread at a density of 10⁵ cells cm⁻² on agar containing 5 mM cyclic AMP, the stalk-cell induction frequencies in strains V-12 M2, NC4, P4 and Ax-2 were 93, 4, 10 and 2%, respectively. At a density of 10⁴ cells cm⁻² and on 1 mM cyclic AMP, V-12 M2 produced 24% stalk cells, whereas the other three strains did not produce any. No stalk cells were observed in controls in the absence of cyclic AMP at 10⁴ cells cm⁻² or in controls at 10⁵ cells cm⁻² where the cells were overlain with Cellophane to prevent fruiting.

We have examined stalk-cell induction in strain V-12 M2 over a range of cell densities from 80 to 10⁵ cells cm⁻² (a density at which aggregation occurs on the control plates but not on those containing 1–5 mM cyclic AMP). The results are shown in Fig. 1. At cell densities greater than 4 × 10⁴ cells cm⁻², almost all of the initially vegetative amoebae were transformed in 2–3 d into stalk-like cells, as judged by their morphology under phase-contrast and in electron micrographs, as well as by their staining with the cellulose-specific indicator calcofluor⁴. The frequency of stalk-cell induction decreased quite rapidly as cell density was reduced, reaching a basal level of approximately 3% below 500 cells cm⁻². At high cell densities, a considerable proportion of the initially separate cells were present in small clumps after 2–3 d incubation and this proportion declined as cell density was lowered.

At cell densities between 5 × 10³ and 2 × 10⁴ cells cm⁻², 70–100% of the cells in small clumps became stalk cells, whereas only 30% of the single cells on the same plates were induced. This latter figure is in turn much higher than the value of 3% observed at very low density (Fig. 1). It therefore seems that cyclic AMP can induce differentiation of isolated amoebae

Table 1 Enhancement of stalk-cell differentiation in isolated cells by a diffusible factor from cells differentiating at high density

Tester cell density (no. of determinations)	% Stalk-cell differentiation			
	Tester cells alone		Tester cells plus high density helper cells	
	Control	Cyclic AMP	Control	Cyclic AMP
10^3 cells cm^{-2} (3)	1.3	4.3	0	80.0
2×10^3 cells cm^{-2} (2)	0	1.3	0	39.6
5×10^3 cells cm^{-2} (1)	0	4.5	0	79.6

Cells were prepared and spread on 1% agar containing 1% Bonner's salts with or without 5 mM cyclic AMP as described in Fig. 1. The first layer of cells was overlaid with Cellophane and, where appropriate, a second layer of cells. In some experiments buffer (5 mM MES, pH 6.0) was included in the agar to exclude the possibility of a pH artefact. This buffer has no effect on the induction process. After 2–3 d, a coverslip was placed on the upper layer of cells and the dishes examined by phase contrast microscopy. Stalk cells were identified by their vacuolated morphology, the remaining cells appearing amoeboid. Induction in the high density population was essentially 100% in all cases. Cellophane 325P (British Cellophane) was prepared by boiling in 1 mM EDTA for 15 min followed by extensive washing in distilled water. It was impermeable to Dextran blue 2000 (molecular weight 2×10^6) and to inulin (molecular weight $\sim 5,000$).

into stalk cells, but that the efficiency of conversion is greatly enhanced by some kind of cell interaction.

The density dependence of stalk-cell induction and the enhanced conversion of single cells at high density could be interpreted in terms of a diffusible inducer or of a process of contact-dependent induction. To distinguish between these possibilities, we asked whether cells undergoing stalk cell differentiation at high density could enhance the frequency of stalk-cell formation in a low density population separated from them by a Cellophane membrane. Similar tests of synergism have been used by other workers^{5,6}. The results are shown in

Table 1. The low density populations alone had an induction frequency of 0–5%, whereas the same cells plated over a dense layer of 'helper' cells gave a frequency of 40–80%. In similar experiments we have found that V-12 M2 'helper' cells also increase the efficiency of stalk-cell induction in strain NC4 and its derivatives. These results suggest that cells undergoing stalk-cell differentiation at high density secrete a dialysable factor which is capable of enhancing cyclic AMP-induced differentiation in isolated cells. It is still possible that actual cell contact is required for the synthesis of this factor by the 'helper' cells.

We have isolated a number of developmentally defective mutants of V-12 M2 after nitrosoguanidine or ethylmethanesulphonate mutagenesis and have screened them for stalk-cell inducibility by cyclic AMP. Only two out of seven totally aggregation-defective mutants were inducible. On the other hand, 14 out of 15 mutants which aggregated but either failed to develop further, or gave aberrant or delayed fruits, were inducible by cyclic AMP. This result suggests that certain events involved in the attainment of aggregation competence are essential for stalk-cell induction.

Table 2 Enhancement of differentiation of *sci-1* at low density by high density wild-type or *sci-1* cells

Cell density and genotype		% Differentiated cell type observed			
Above Cellophane	Below Cellophane	Above Cellophane Stalk	Below Cellophane Spore	Above Cellophane Stalk	Below Cellophane Spore
5×10^3 cm^{-2} mutant	None	4.3	0.4	—	—
5×10^3 cm^{-2} mutant	2.5×10^5 cm^{-2} mutant	43.3	8.1	39.5	12.6
5×10^3 cm^{-2} mutant	2.5×10^5 cm^{-2} wild type	27.0	8.9	79.3	0

Cells of the mutant (*sci-1*, clone 5) and wild type were collected as described in Fig. 2. Cells of either type were plated at high density directly on to agar (2 ml 1.5% agar containing 1% Bonner's salt solution, 5 mM cyclic AMP, 250 $\mu\text{g ml}^{-1}$ streptomycin in a 5-cm diameter dish). Cellophane (325P) was placed on top and a low density of *sci-1* cells spread on it. In control plates the high density cells were omitted. After 2–4 d at 22 °C a coverslip was placed on the upper layer of cells and the cells scored above and below the Cellophane. Results are means of three experiments.

Because stalk cells are non-viable⁷ and our induction procedure is efficient, we have been able to use the cyclic AMP induction procedure to enrich for resistant mutants in a mutagen-treated population. These experiments were carried out using an acriflavine-resistant mutant of V-12 M2 which gives reasonably compact plaques when plated with *Klebsiella aerogenes* on medium containing acriflavine. The majority of the non-inducible mutants isolated by this procedure show aberrant development, most being aggregation defective. During the screening of colonies surviving the selection procedure, we encountered one unusual mutant (*sci-1*) in which cyclic AMP induced the formation of spores—identified by their morphology and survival after exposure to heat⁸ or detergent (R. R. Kay, unpublished and ref.9). Figure 2 shows the pattern of differentiation of cells of *sci-1* at various densities under Cellophane in the presence and absence of cyclic AMP. In the presence of cyclic AMP the mutant formed both stalk cells and spores. The formation of both was density dependent; however, stalk-cell differentiation was relatively more efficient at low density and was reduced at high density. In the absence of cyclic AMP, few spores were produced at any density, whereas the frequency of stalk-cell differentiation reached a maximum at 5×10^4 cells cm^{-2} and then declined. The mutant cells incubated at low density in the presence of 5 mM cyclic AMP could be induced to differentiate into both stalk and spore cells by a high density of either *sci-1* or wild-type V-12 M2 cells (Table 2).

In normal developmental conditions the mutant produces

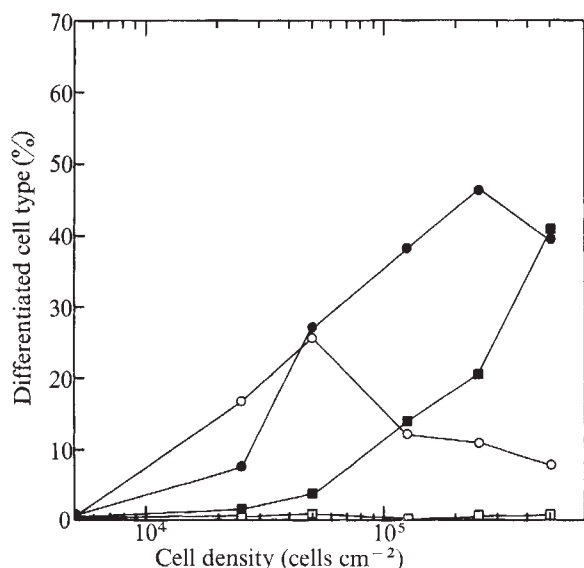


Fig. 2 Formation of stalk and spore cells by mutant *sci-1* under Cellophane. *Sci-1* cells were collected from growth plates in KK_2 buffer (16.5 mM KH_2PO_4 , 3.8 mM K_2HPO_4 , 2 mM MgSO_4 , pH ~ 6.2) with 60 mM KCl added and pelleted at 200g for 5 min. After three further washes in KK_2 and one in 1% Bonner's salts solution, the cells were spread at the stated densities on 2 ml 1.5% agar (Oxoid L28) containing 1% Bonner's salts, 250 $\mu\text{g ml}^{-1}$ streptomycin with or without 5 mM cyclic AMP, in a 5-cm diameter Petri dish. Cellophane was then placed over the cells and after 2–4 d incubation at 22 °C, stalk and spore cells were scored microscopically. At least 200 cells were scored on each plate and the results given are the average of five separate experiments. Two of these experiments were carried out on the original isolate of the mutant and three on clone 5 derived from it. ■, Spore+cyclic AMP; ●, stalk+cyclic AMP; □, spore, no cyclic AMP; ○, stalk, no cyclic AMP.

pyramidal fruits, sometimes with a bulbous tip, composed of jumbled patches of stalk and spore cells. Similarities between the fruit of *sci-1* and the mutant *Fr17* previously isolated by Sonnenborn *et al.*^{10,11} prompted us to examine the behaviour of *Fr17* under Cellophane. Vegetative cells of *Fr17* at low density (5×10^3 cells cm^{-2}) placed under Cellophane produced less than 1% stalk or spore cells, with or without cyclic AMP. At high cell densities (5×10^5 cells cm^{-2}), however, it produced 60–70% spores and 1–10% stalk cells in the presence or absence of cyclic AMP.

We are at present attempting to purify the putative factor(s) that enhances stalk-cell induction by cyclic AMP. The factor seems to be stable since cell-free medium collected after stalk-cell induction in dense cells increases the yield of stalk cells in low density populations. Knowledge of the identity of the factor(s) should be helpful in elucidating its role (if any) in normal development, and will also enable us to test whether the same factor is implicated in the density-dependent differentiation of stalk cells and spores in mutants *sci-1* and *Fr17*. In any event the use of a Cellophane overlay to examine cell differentiation in the absence of normal morphogenesis should facilitate further analysis of the interplay of elements responsible for pattern formation in *D. discoideum*.

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Somatic hybridisation of *Penicillium roquefortii* with *P. chrysogenum* after protoplast fusion

FUSION of fungal protoplasts after treatment with polyethylene glycol (PEG) has been described as a new technique for intraspecific heterokaryon formation at high frequency^{1–3}. We now report the formation of interspecific somatic hybrids between *Penicillium roquefortii* and *P. chrysogenum* by PEG-induced fusion of their protoplasts.

Stable auxotrophic mutants of *P. roquefortii* (University of Nottingham Collection) and *P. chrysogenum* Wis. 49-2105 were obtained after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine⁴. Symbols designating the requirements of the mutant strains and the mutant spore colour are as follows: *met/cys/glu/val* (methionine or cysteine or glutamine or valine); *met* (methionine); *trp* (tryptophan); *lys* (lysine); *arg* (arginine); *leu* (leucine); *lys pro/arg* (lysine and proline or lysine and arginine); *leu met/cys* (leucine and methionine or leucine and cysteine); *whi* (white spore colour); *ylo* (yellow spore colour). Strains were maintained and allowed to sporulate on a complete medium (CM)⁵. Mycelium for protoplast formation was grown in a production medium (PM), consisting of inorganic minimal medium (MM)⁵ supplemented with 0.2% Difco yeast extract and the requirements of the auxotrophs. Protoplasts were produced as described previously⁵ and fusion was carried out as for intraspecific protoplast fusion^{1,3}. Suitable dilutions were plated on to hypertonic MM to select fused protoplasts of nutritionally complementary strains. Surviving protoplasts were counted on hypertonic PM.

After PEG treatment of a mixture of complementing *P. roquefortii* and *P. chrysogenum* protoplasts, small prototrophic colonies developed on MM. No growth was observed on MM when protoplasts of the two strains were mixed without PEG or when they were treated separately with PEG and then mixed. Spores could not be fused and did not germinate on MM in any of the conditions tried. These results indicated that the prototrophic colonies arose from balanced fused protoplasts of *P. roquefortii* and *P. chrysogenum* and that they were not the result of back mutation or cross feeding between unfused protoplasts. Fusion frequency was 0.03–3.4% (Table 1). These yields were similar to those obtained for PEG-induced intraspecific protoplast fusion^{1,3}. Pure lines of interspecific hybrids were established by repeated cloning of single protoplasts produced from mycelium grown in MM shake cultures inoculated with prototrophic mycelial fragments. After plating on MM, single protoplasts reverted to prototrophic colonies and this procedure could be repeated indefinitely.

The colonies of the interspecific hybrids were morphologically different from the parental strains and they could be classified into three main types (Fig. 1). On MM, type 1 colonies were slowly growing with limited hyphal branching and failed to sporulate. On CM, on PM or on MM supplemented with the requirements of either one of the parental strains, they grew faster and produced parental auxotrophic *P. roquefortii* spores. *P. chrysogenum* spores, however, were never observed. Type 1 colonies predominated in the first culture after fusion treatment and when protoplasts were prepared from 6-d-old MM cultures of type 1 mycelial fragments. On the other hand, when type 1 mycelium was incubated for 10–12 d, more protoplasts developed into type 2 colonies, consisting of a loosely meshed network of broadly spreading hyphae. Like type 1 colonies, they did not sporulate on MM, and they produced only *P. roquefortii* auxotrophic spores on CM or PM. Protoplasts from type 2 mycelium reverted to type 2 colonies, irrespective of the age

Table 1 Percentage of fusion between protoplasts of *P. roquefortii* and *P. chrysogenum* complementing auxotrophs

<i>P. roquefortii</i> auxotrophs*	<i>lys pro/arg whi</i>	<i>leu met/cys ylo</i>	<i>P. chrysogenum</i> auxotrophs*	<i>leu</i>	<i>arg</i>
<i>met/cys/glu/val</i>	1.60	1.25	2.00	0.80	0.60
<i>met</i>	0.45	0.30	NT	NT	NT
<i>trp</i>	0.31	0.03	0.24	3.40	0.18

NT, Not tested.

Protoplasts, produced by treatment (27 °C, 4 h) of 20-h-old mycelium with *Oxytropis* cellulase (5 mg ml^{-1}) and Strepzyme 12,705 (10 mg ml^{-1}) in 0.8 M MgSO_4 (for *P. roquefortii*) or 0.6 M NaCl (for *P. chrysogenum*) were separated from mycelial debris by filtration through a sintered glass filter (porosity 1). Fusion was achieved by mixing washed protoplasts (10^5 of each auxotroph), treating them (30 °C, 10 min) with 1 ml of 30% (w/v) PEG molecular weight 6,000 in 0.01 M CaCl_2 and 0.05 M glycine adjusted to pH 8.0 with NaOH, and eliminating PEG by three washes in 0.8 M NaCl. Protoplasts were plated on to MM and PM to determine percentage of fusion.

*About 10% of the protoplasts regenerated on PM after fusion treatment³.

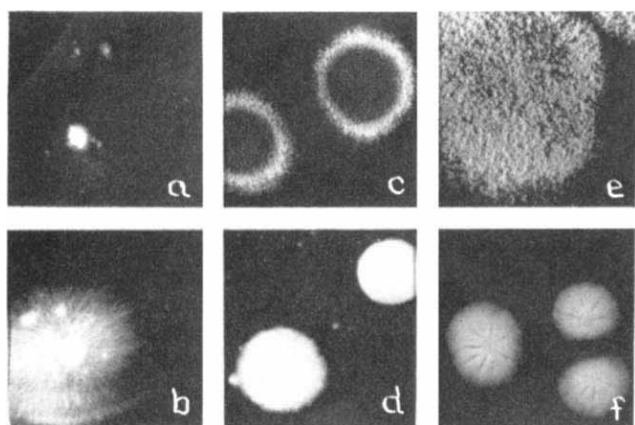


Fig. 1 Different types of colonies after reversion of protoplasts of *P. roquefortii* met/cys/glu/val, *P. chrysogenum* lys pro/arg whi and their interspecific somatic hybrids. *a*, Type 1 colonies on MM; *b*, type 2 colonies on MM; *c*, sporulating type 1 colonies on MM supplemented with methionine; *d*, type 3 colonies sporulating on MM; *e*, parental *P. roquefortii* met/cys/glu/val on MM supplemented with lysine and proline; *f*, parental *P. chrysogenum* lys pro/arg whi on MM supplemented with lysine and proline.

of the mycelium. Colonies with compact mycelium and conidiophores branched similarly to *P. chrysogenum* were designated type 3. They appeared, usually less than 0.1% of the total, among type 1 and type 2 colonies after reversion of protoplasts from type 1 or type 2 mycelium. Type 3 colonies sporulated even on MM and released greenish-white prototrophic spores with a larger diameter than those of the parental strains (Table 2). Single spore isolates and protoplasts again developed into type 3 colonies. Occasionally, the latter colonies showed sectors of *P. roquefortii* spores.

The ability of type 1 and type 2 mycelia to release parental *P. roquefortii* spores showed that *P. roquefortii* nuclei were present in the interspecific mycelium. On the other hand, the prototrophy as well as the ability of type 1 and type 2 mycelium to revert to *P. chrysogenum*-like type 3 colonies indicated that at least part of the *P. chrysogenum* genome was present. Further evidence for this was obtained from studies of penicillin production by the interspecific progeny (Table 2). When grown in a rich fermentation medium⁶ or in the medium of Jarvis and Johnson⁷

(JJ), mycelium of all colony types produced antibiotic substances, which were inhibitory to *Sarcina lutea* ATCC 9341 and to a penicillin-sensitive *Staphylococcus aureus* ATCC 6558 P, but not to a penicillin-resistant *Staphylococcus*. The antibiotic activity disappeared on treatment with penicillinase. Paper chromatography⁸ and gas-liquid chromatography⁹ showed that the antibiotics were a mixture of benzyl penicillin, *n*-amyl penicillin and 2-pentenyl penicillin. When grown in a suitable medium⁶, the parental *P. chrysogenum* lys pro/arg whi produced a similar mixture of penicillins. But growth of this strain, either alone or cocultivated with *P. roquefortii*, was completely inhibited by the ammonium lactate and ammonium acetate in JJ medium. In contrast, the interspecific hybrids and *P. roquefortii* grew well in JJ medium, but neither the *P. roquefortii* culture filtrates nor mycelial extracts contained any antibiotic substance when assayed against *S. lutea* or *St. aureus*. Thus it seems unlikely that the penicillins in the cultures of the interspecific mycelium were produced by segregated parental *P. chrysogenum*. Moreover, the latter was never re-isolated.

Although conclusive evidence is lacking, several observations indicated that mycelium from type 1 and type 2 colonies was heterokaryotic, whereas type 3 mycelium might be diploid. Type 1 and type 2 colonies were prototrophic and produced penicillins, but released parental *P. roquefortii* spores. Moreover, growth and sporulation were stimulated by addition of the amino acids required by either of the parental strains (Table 2), indicating that the nuclei of both complementing strains were still present. Type 3 colonies produced penicillins and formed prototrophic spores with a larger diameter. Furthermore, they sometimes showed sectors of haploid *P. roquefortii* segregants. Those characteristics obviously suggest diploidy. It is, however, still unclear why only *P. roquefortii* and not *P. chrysogenum* could be re-isolated. *P. roquefortii* might be dominant over *P. chrysogenum* for certain alleles and could have repressed the formation of *P. chrysogenum* spores. On the other hand, it cannot be excluded that *P. chrysogenum* should have lost chromosomal material. Loss of chromosomes is a common phenomenon after somatic fusion of mammalian cells^{10,11}.

The present studies demonstrate that somatic hybrids of related species of *Penicillium* can be produced by PEG-induced fusion of their protoplasts. Whether it will be possible to obtain viable hybrids of less related species is under investigation.

We thank P. Adriaens and B. Meesschaert for chromatographic determination, Dr J. F. Peberdy for discussion and

Table 2 Properties of *P. roquefortii* met/cys/glu/val; *P. chrysogenum* lys pro/arg whi and their interspecific somatic hybrids

	<i>P. roquefortii</i> met/cys/glu/val	<i>P. chrysogenum</i> lys pro/arg whi	Interspecific heterokaryon of met/cys/glu/val + lys pro/arg whi (type 1 colonies)	Supposed diploid of met/cys/glu/val + lys pro/arg whi
Spores				
Colour	Bright green	White	Dark green*	Greenish-white
Size (µm)	3.35 ± 0.50	3.67 ± 0.62	3.44 ± 0.44*	4.96 ± 0.31
Auxotrophy	Methionine or cysteine or glutamine or valine	Lysine and proline or lysine and arginine	Methionine or cysteine or glutamine or valine	Prototrophic
Colony morphology				
Type	Loose	Very compact	Intermediate compact	Very compact
Diameter (mm) after 8 d (27 °C) in:				
MM	No growth	No growth	3.78 ± 0.86	7.70 ± 1.70
MM, supplemented with methionine	8.0	No growth	5.31 ± 0.86	7.60 ± 1.0
MM, supplemented with lysine and proline —or lysine and arginine	No growth	9.0	5.96 ± 1.71	7.50 ± 1.4
MM, supplemented with leucine and alanine	No growth	No growth	4.24 ± 1.40	NT
Penicillin production (as µg benzyl penicillin per ml) in:				
Rich fermentation medium	0	32.0	1.8	± 0.1†
JJ	0	No growth	0.30	± 0.1†

*On MM, supplemented with methionine or with lysine and proline; no spores were formed on unsupplemented MM.

†Penicillins were partly inactivated by alkalisation of the medium.

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Molecular imaging of dislocations in 16 chlorocopper phthalocyanine

DISLOCATIONS in molecular crystals are well known, but have been studied on the microscale chiefly with a view to establishing their role in phase changes¹ or topotactic transformations². Here, the positions of individual molecules can be identified, and, in so far as 16 chlorocopper phthalocyanine is a typical molecular crystal, the results are important for concepts of dislocations and intercrystalline boundaries in such crystals.

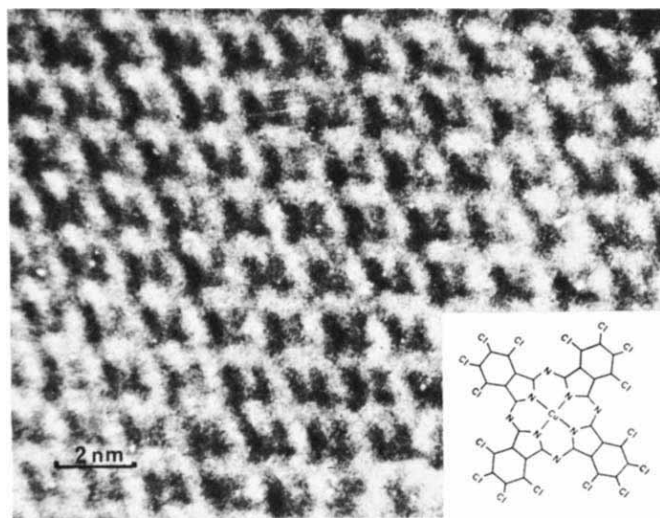


Fig. 1 Perfect crystal of 16 chlorocopper phthalocyanine lying on (001) face.

Figure 1 shows an area of a perfect crystal and illustrates some facets of the electron microscopy used for imaging. First, the crystalline film was prepared epitaxially on KCl at 200 °C at a maximum thickness < 20 nm. The film was then supported on a dimpled micromesh specimen grid—dimpled to provide a varied orientation of the specimen film, rather than using a tilting stage, with its commensurately poorer resolution. The film was lying on the (001) face and as Fig. 2 shows, the specimen had to be tilted, so that the columns of molecules are parallel to the electron beam³. This material, like all molecular crystals, is very sensitive to electron radiation damage, so exposure to the electron beam must be minimised. Ilford Industrial G X-ray film was used to record the image, with a very short exposure time.

With this specimen thickness and for this material in this

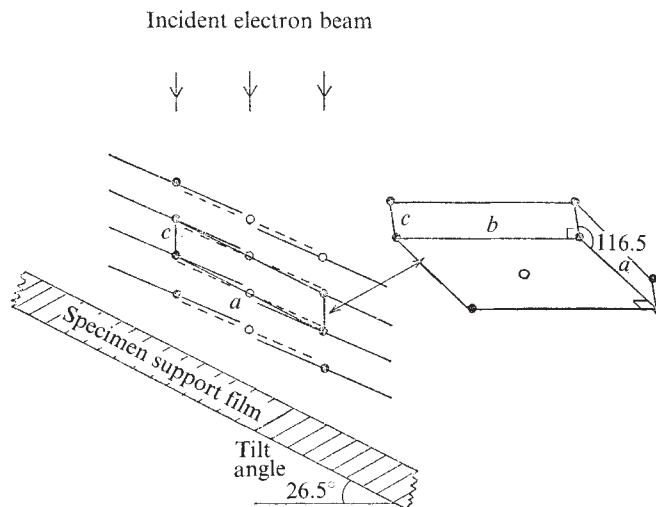


Fig. 2 Projection of molecular stacking along *b* axis showing required specimen tilt for molecular imaging, and geometry of unit cell. (Space group *C2/c*) ●, Copper atom at corners; ○, copper atom at face centre.

orientation, dynamical imaging effects should be absent and computed images^{4,5}, allowing for instabilities of lens constants and for partial coherence, showed that 80 nm was the optimum defocus position. Also, for this size of molecule rapid oscillations of the phase contrast transfer functions do not occur within 20 nm of the optimum defocus position, so there should not be significant contrast change within the thickness of the film. In the light of present knowledge of electron optical imaging, therefore, we believe that the images represent the structure of the specimen.

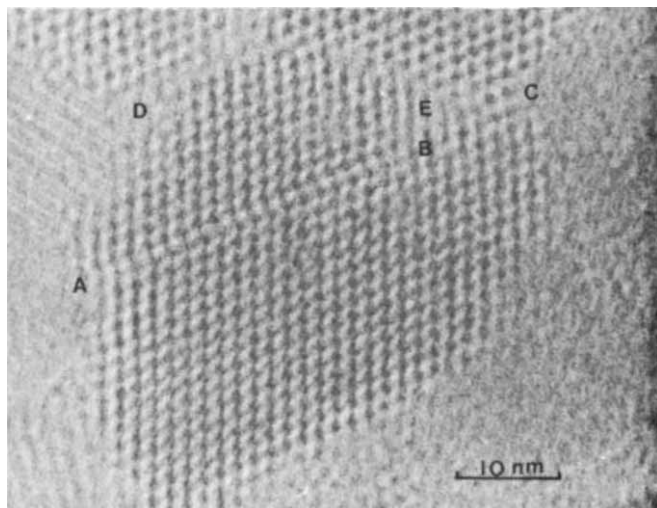
Many dislocations were observed, but since the crystallite size was small (< 2,000 nm²) the internal dislocation density was small and was not statistically significant compared with the extent of grain boundaries. Dislocations of all types were present and examples of each will be discussed.

Figure 3 shows a dislocation A–B which, at first sight, suggests a tilt or twin system, but since the contrast on either side of A–B is similar, the vertical orientation of the columns of molecules on either side must be similar; a screw dislocation of

vector $\frac{b}{2} \langle 110 \rangle$ would explain the image. There is a contrast

change at B that could be ascribed tentatively to the interaction of this screw dislocation with the strain field of a mainly edge

Fig. 3 Imperfect region of crystal showing line dislocations and boundaries.



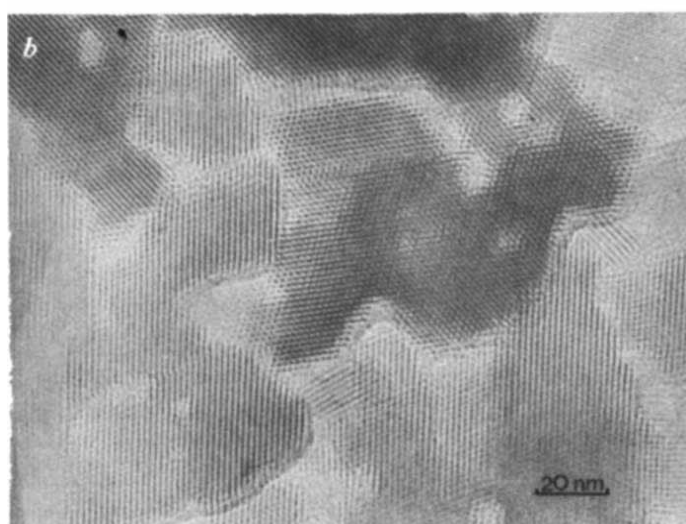
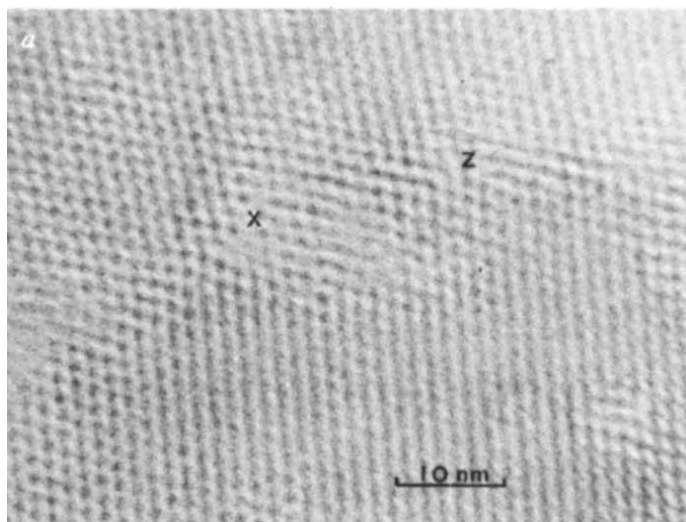


Fig. 4 *a*, Imperfect region of crystal showing column vacancies and other dislocations. *b*, Crystal boundaries showing distortion of peripheral molecular stacking, to reduce the boundary strain energy.

dislocation situated at C, but the presence of the boundary, DE, prevents more accurate identification. DE may be described as a grain boundary in spite of the close geometric relationship across the boundary which is a feature of these crystals.

Figure 4*a* shows a region of distortion with an apparent vacancy at X. This corresponds to the absence of a column of molecules, since even half a column would still have significant contrast in spite of its different defocus position. To the right of the vacancy, there is some tilting of the crystal as shown by the absence of images of discrete molecular columns, but a clear boundary between the two regions of the crystal cannot be determined. Individual dislocations—for example, in region Z—can be distinguished, but they are notable for both their small cores and small strain fields, although the dislocations may be affected by the presence of the boundary. Similar accommodation of distortion is also shown for the grain boundaries in Fig. 4*b*.

It is this lack of definition of boundaries and high distortion that is unexpected in these micrographs. We had anticipated that crystallites would be isolated entities, and that there would be a well defined strain field around each dislocation and internal boundary. In fact, neither is evident; the grain boundaries are not clearly defined, and there has been considerable molecular mobility at boundaries to reduce strain. The accommodation of considerable distortion in these crystals therefore suggests that molecular orientation is not sharply defined in these molecules and their effective radii can vary considerably. This is important

for the concept of minimum-energy boundaries and implies that polycrystalline aggregates have a more intimate relationship than has hitherto been thought.

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Models for the bacterial iron-transport chelate enterochelin

MICROBIAL iron-transport compounds, or siderochromes are of two general structural types, the phenolates and the hydroxamates^{1,2}. X-ray studies of several of the latter, for example, ferrichrome A (ref. 3), ferrioxamine E (ref. 4) and mycobactin P (ref. 5), establish the anion of hydroxamic acid ($-\text{N}(\text{O}^-)\text{CO}-$) as the dominant metal-binding moiety, with discrete, neutral $[\text{FeO}_6]$ (refs 3 and 4) or $[\text{FeO}_5\text{N}]$ (ref. 5) units being involved. No similar structural data are at present available, however, for any member of the phenolate class and there is some ambiguity about the metal-binding sites. This is demonstrated for the most widely studied member of the group, enterochelin (or enterobactin), in Fig. 1. Both modes of attachment have parallels in the well known colour reactions of iron(III) with phenols, polyphenols and catechols and in the strong coordination of deprotonated amide nitrogen in simple peptide complexes of the transition elements⁷. Furthermore, molecular models (Dreiding or CPK) of iron (III) enterochelin can readily be constructed with either bonding combination. Here we report our studies of two catechol (1,2-dihydroxybenzene) complexes containing the coordination type given by Fig. 1*a* and compare some of their properties with those of iron(III)-enterochelin (part of this work was presented in ref. 8).

Following Selles⁹, two complexes were crystallised from ethanol or ethanol-water mixtures containing ferric acetate, catechol and piperidine. Conditions of low base yielded deep purple crystals, whereas excess piperidine resulted in burgundy-red crystals. X-ray data for both compounds were recorded with graphite-monochromated $\text{CuK}\alpha$ radiation ($\lambda = 1.5418 \text{ \AA}$), using a Picker-FACS I four-circle diffractometer and a θ - 2θ scan technique.

Crystals of the purple racemic piperidinium μ -acetato-di- μ -1,2-benzenediolato-bis-1,2-benzenediolato-ferrate(III), $(\text{C}_5\text{H}_{12}\text{N})_3[(\text{CH}_3\text{COO})\{\text{Fe}(\text{C}_6\text{H}_4\text{O}_2)_2\}_2]$ are tetragonal, space group $P4$, with $a = b = 27.593(3)$, $c = 12.234(2) \text{ \AA}$, $Z = 8$, $D_m = 1.23$, $D_c = 1.227 \text{ g cm}^{-3}$. The crystals showed a rapid decrease in reflection intensity with increasing scattering angle and only 2,467 unique reflections of some 16,000 collected had intensities greater than $3\sigma(I)$. The structure was solved by Patterson and Fourier techniques, and refined by large-block least-squares methods, giving $R = 0.092$. At the present stage of refinement, hydrogen atoms have not been included in the scattering model and although the correctness of the structure is not in doubt, the precision of the final result will be limited by the unfavourable data-parameter ratio and by the apparent

disordering of one piperidinium cation. Two crystallographically distinct dimeric units of similar structure are present in the asymmetric unit together with piperidinium cations. As Fig. 2a shows, the two $[\text{FeO}_6]$ octahedra share a common edge using oxygens of two catechol dianions and adjacent apical coordination sites are bridged by an acetate ion. Each octahedron contains one bidentate catechol moiety which is not involved in bridging the metal atoms. The Fe–O bond lengths are in the range 1.94–2.08 Å, the O–Fe–O chelate ring angles are in the range 82.5–85.1°, with no apparent distinction between terminal and bridging chelates at the present stage of refinement, and the mean Fe–Fe distance is 3.15 Å for the two discrete dimer units. With the acetate considered to occupy the apical positions, the interplanar angle for the two basal planes is 150°. One piperidinium cation forms two intramolecular hydrogen bonds, whereas the other two cations bridge neighbouring 4-related anions. The overall structure can be described as rosettes of associated cations and anions, each containing four dimeric units arranged about the crystallographic 4 centres.

The burgundy-red crystals of the high base form have the composition $(\text{C}_6\text{H}_{12}\text{N})_3[\text{Fe}(\text{C}_6\text{H}_4\text{O}_2)_3] \cdot \text{H}_2\text{O}$ and belong to the monoclinic space group $C2/c$ with $a = 22.887(4)$, $b = 11.747(1)$, $c = 23.999(3)$ Å, $\beta = 118.19(1)^\circ$, $Z = 8$, $D_m = 1.29$, $D_c = 1.282 \text{ g cm}^{-3}$; 6,320 X-ray reflections were collected of which 3,422 had $I > 3\sigma(I)$. The coordinates of all non-hydrogen atoms were determined using the direct phasing programme MULTAN¹⁰ followed by several cycles of structure factor/difference Fourier calculations. Hydrogen atoms were positioned by calculation (O–H not included) and anisotropic refinement by full matrix least-squares methods resulted in a conventional R value of 0.054. The structure contains the expected 'propeller' of three bidentate catechol dianions, providing the octahedral coordination geometry $[\text{FeO}_6]^{3-}$ (Fig. 2b). Fe–O bond lengths vary between 1.991 and 2.036(8) Å, with a mean value of 2.020 Å. The O–Fe–O angles of the five-membered chelate rings, 81.1, 82.3 and 80.2(3)°, and the mean twist angle about the threefold axis, 46.4° (60° for a regular octahedron, 0° for a trigonal prism), reflect significant distortions from regular octahedral geometry. In the analogous potassium salt recently reported¹¹ the mean Fe–O bond length is 2.015(6) and the mean values of the O–Fe–O and twist angles are 81.26(7) and 44.7°, respectively. Considerably less distortion is found in the *tris*-catechol complexes of Cr(III) (83.56, 50.5°) (ref. 11), As(V) (88.2, 55.2°) (ref. 12) and P(V) (91.4, 54.9°) (ref. 13).

A comparison of the spectroscopic and magnetic properties given in Table 1 suggests that the monomeric, but not the dimeric, iron(III)–catechol complex is an appropriate model for the coordination geometry in iron(III)–enterochelin. Furthermore, these data suggest a similar electronic environment for the monomer and iron(III)–enterochelin. The ESR and Mössbauer data for the monomer are generally consistent with those reported for other iron-transport compounds but there are some differences in detail. Thus at 4.2 K in small applied fields (~2 koersted) the Mössbauer spectrum of the solid monomer does not display the degree of fine structure exhibited by that of methanol solutions of iron(III)–enterochelin¹⁴. The hyperfine field is also somewhat smaller in the monomer. Oosterhuis has recently given a detailed analysis of the ESR and Mössbauer

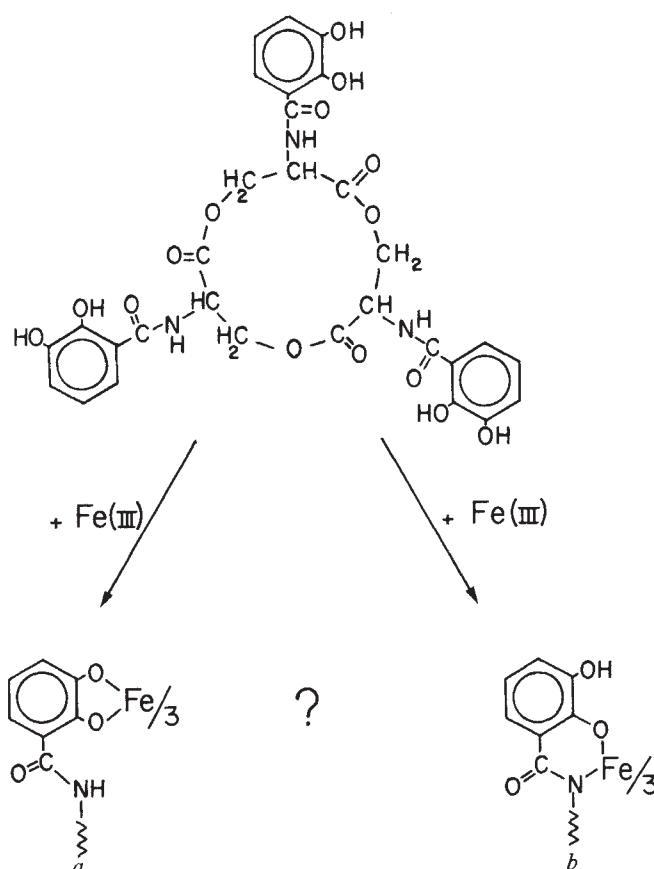


Fig. 1 Structure of enterochelin, the cyclic triester of 2,3-dihydroxy-N-benzoyl-L-serine⁸, and two possible coordination geometries with iron(III). Coordination by way of the six catechol oxygens leads to a $[\text{FeO}_6]^{3-}$ unit containing three five-membered chelate rings (a), whereas coordination of a phenolate oxygen and a deprotonated amide nitrogen of each serine residue gives rise to a $[\text{FeO}_5\text{N}]^{3-}$ environment and six-membered chelate rings (b). Although the $[\text{FeO}_6]^{3-}$ coordination type has been proposed by several workers^{1,6}, it should be pointed out that all known phenolate siderochromes contain amide linkages to amino acids or amino acid fragments⁴.

spectra of iron transport compounds in terms of a rhombically distorted electronic model¹⁵. The crystal structure of the monomer certainly exhibits small variations in bond lengths and bond angles about the metal and in the pattern of hydrogen bonding to the phenolic oxygen atoms. The ESR spectrum for the dimer is complex showing transitions ranging from about $g = 5$ to 1, and the Mössbauer and magnetic susceptibility data are similar to those observed¹⁶ for other exchange-coupled iron(III) dimers which normally have J values of the order of 10 cm^{-1} (ref. 6). Also all three complexes liberate three equivalents of Na^+ ion from Sephadex C-25 cation exchange resin from which they can be eluted with unchanged visible spectra. This apparent robustness in solution and in the solid state¹⁷ together with the physical properties given in Table 1 strongly suggest a monomeric *tris*-catecholate coordination environment (Fig. 1a) for iron(III)–enterochelin.

Table 1 Spectroscopic and magnetic properties of iron(III)–enterochelin and iron(III)–catechol complexes

	$[\text{Fe(III)-enterochelin}]^{-8}$	$[\text{Fe(catechol)}]^{3-}$	$[(\text{acetato})\{\text{Fe(catechol)}_2\}_2]^{3-}$
$\lambda_{\text{max}}(\text{nm})$ (ε/Fe)	496(5,600) (ref. 6)	496(4,700)	570(3,400)
ESR (<i>g</i> values)*	4.3 (ref. 6)	4.3	Complex (see text)*
Mössbauer (hyperfine† coupling constant, koersted per unit spin)	222 (ref. 15)	201	~0
Magnetic susceptibility‡	$S = 5/2$	$S = 5/2$ §	$S_1 = S_2 = 5/2$ § $J \approx 10 \text{ cm}^{-1}$

* Data collected on frozen aqueous solutions at temperatures close to 77 K.

† Data collected at 4.2 K (ref. 16). The hyperfine field was resolved in an applied field of 52 koersted parallel to the γ-ray direction.

‡ Data collected from 300–4.2 K as described elsewhere¹⁷.

§ P. D. W. Boyd and R. L. Martin, unpublished data.

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Erratum

In the article "Effect of phloridzin and phloroglucinol on apple shoots" by O. P. Jones (*Nature*, **262**, 392; 1976) Figs 1 and 2 have been transposed. The legends are correct as they stand. In Table 1 the column headed 'Medium' should read

1-IAA
1-IAA+PZ
1
1+PZ
1
1+PZ
1
1+PG

and not as printed.

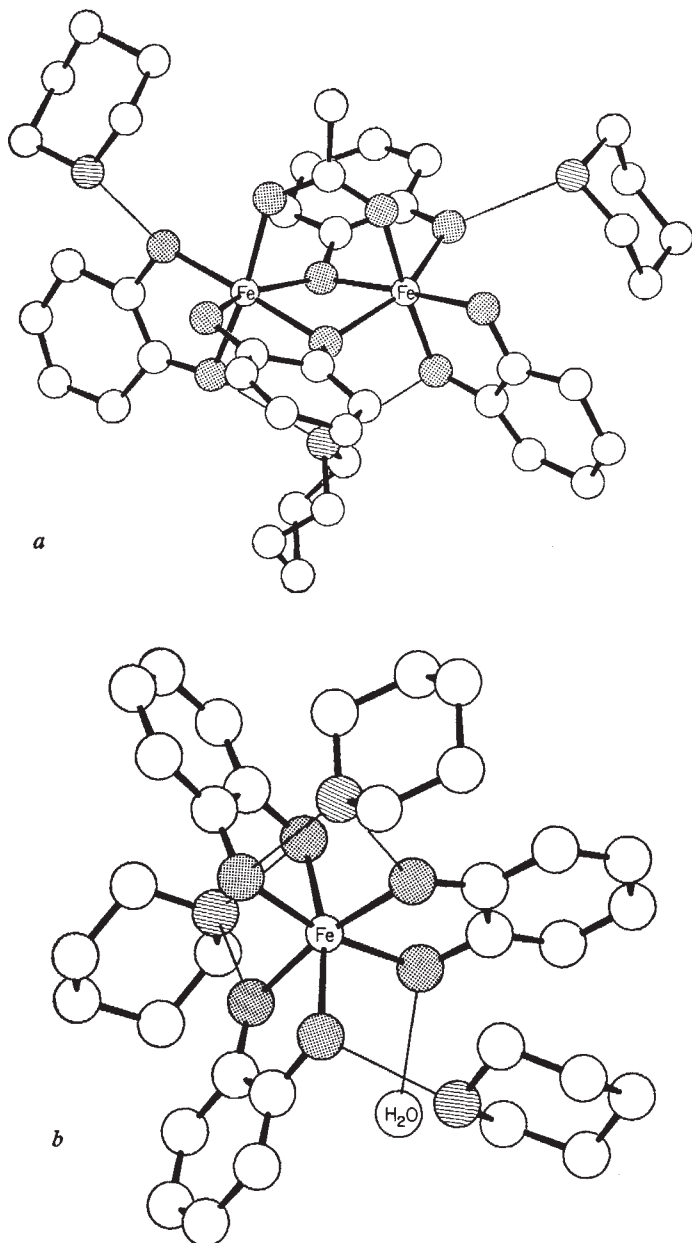


Fig. 2 *a*, Structure of one of the $(C_5H_4N)_3[(CH_3COO)_2Fe(C_6H_4O_2)_2]_2$ units. *b*, Structure of the molecular unit of $(C_5H_4N)_3[Fe(C_6H_4O_2)_3] \cdot H_2O$, in which each phenolate oxygen is hydrogen-bonded to a C_5H_4N cation or H_2O molecule. In both figures N atoms are indicated by lined circles, O atoms by dotted circles, and hydrogen bonds by lines. Hydrogen bonds between molecular units in the crystals are not shown.

We thank Professor F. Gibson and Drs I. G. Young and I. G. O'Brien for assistance in preparing enterochelin and for other helpful advice. Iron(III)-enterochelin has been isolated from aqueous solution as the Ph_4As^+ or Ph_3MeP^+ salts. These compounds are very soluble in $CHCl_3$, CH_2Cl_2 and acetone, and may be readily converted to other salts by titration with the appropriate perchlorate salt (T. Rauchfuss, unpublished).

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matters arising

B-emission stars and X-ray sources

IN 1975, an hypothesis of the binary nature of B-emission (Be) stars was proposed^{1,2}. Referring partly to this and to the observed association of some X-ray transients with Be stars, Maraschi *et al.*³ suggested that the classical Be stars, as the optical counterparts of the X-ray transients, could be responsible for the X-ray bursts of these objects. We present here some arguments against this interpretation.

Be stars are essentially normal, rapidly-rotating B stars situated (on average) about one magnitude above the main sequence in the Hertzsprung–Russell diagram. They are enclosed by an extended envelope producing emission (and sometimes also sharp absorption) lines in their spectra. The envelope lines usually exhibit several kinds of variability (for review see refs 1, 2): (1) long term profile and velocity variations, including sometimes even complete disappearance and reappearance of the envelope lines within 1–10 yr; (2) periodic velocity and profile variations with typical periods of 10–100 d; (3) rapid profile variations taking place within several hours or minutes. The main idea of the binary hypothesis of the Be phenomenon is that the Be envelope is formed as an accretion disk enclosing the mass-gaining components of certain mass-exchanging systems and that the high rate of rotation of Be stars is induced by infalling matter. Periodic velocity and profile variations are interpreted as reflecting the orbital motion while the long term changes are understood as consequences of varying rate of mass transfer between components (similar to the Algol binaries). This view is supported by observational data. In every known case, the basic properties of the Be stars recognised as binaries agree well with the theoretical predictions.

Maraschi *et al.*³ suggested that the X-ray transients represent some later evolutionary stage of the Be binaries after mass exchange. In other words, they implicitly assume that the Be envelope once formed is a long lived phenomenon lasting even after the original mass-losing component has evolved to a compact object. They then interpret the X-ray bursts as a consequence of occasional infalls of

matter from the Be on to the compact component controlled by some (unspecified) rotational instability of the Be envelope. There is no observational evidence to support this view. The long term variations of Be stars seem to indicate that the envelopes can dissipate rather quickly without the presence of a continuing supply of matter. Also, practically all known Be binaries exhibit clear signs of continuing mass transfer towards the Be component.

A more serious objection exists, however. It is important to realise that two groups of Be binaries are observed. In many cases, it is indeed the mass-gaining star enclosed by an envelope which dominates in the spectrum. Such a system can then appear as a 'single' Be star (ζ Tau, 4 Her or 88 Her can serve as examples). In other cases, however, it is the mass-losing star, which is the brighter of the two. But even then, some emission lines originating in the envelope around the (now invisible) mass-gaining star (or, alternatively, around the whole system) can be observed. If, at the same time, the mass-losing star is of a B type, such an object is often also classified as a Be star. This is the case of Be binaries HD 187399 (ref. 4) and HD 173219 (ref. 5), which are listed in all catalogues of Be stars. HD 72754, β Lyr, W Cru and probably X Per itself belong to the same category. This classification of Be binaries is convincingly documented by observations. Radical velocities of the hydrogen emission lines of all these objects invariably indicate that in any known case the emitting envelope does not enclose the mass-losing star—in clear contradiction to the expectations of the model of Maraschi *et al.*³. The Be stars assumed in that model do not seem to be identical to the Be stars normally observed and only by measuring the emission and absorption velocities of the X-ray transients can the existence of the objects postulated by Maraschi *et al.* be confirmed.

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¹ Kriz, S., and Harmanec, P., *Bull. astr. Inst. Czech.*, **26**, 65 (1975).

² Harmanec, P., and Kriz, S., *IAU Symp.*, **70** (1975).

³ Maraschi, L., Treves, A., and van den Heuvel, E. P. J., *Nature*, **259**, 292 (1976).

⁴ Hutchings, J. B., and Redman, R. O., *Mon. Not. R. astr. Soc.*, **163**, 209 (1973).

⁵ Hutchings, J. B., and Redman, R. O., *Mon. Not. R. astr. Soc.*, **163**, 219 (1973).

Environmental effects on alcohol selection by mice

RANDALL and Lester¹ attempted, by transplanting fertilised ova to mothers of a different strain, to estimate the relative contribution of heredity and environment to alcohol selection by mice. Their experiment was designed to allow the following comparisons of alcohol consumption to be made:

- (1) *a*, Offspring resulting from the transfer of C57BL/6 strain ova to DBA/2 mothers were compared with *b*, offspring from C57 ova transferred to C57 mothers (control transplants) and with *c*, naturally bred (untreated control) C57 mice;
- (2) *a*, Offspring resulting from DBA ova transferred to C57 mothers were compared with *b*, control transplant DBAs and with *c*, untreated control DBA mice.

The absence of a difference between groups *b* and *c* would imply that the transplantation procedure did not affect offspring and then a difference between groups *a* and *b* would imply that the maternal environment affects alcohol consumption of offspring. This pattern of findings is what they report: DBAs transferred to C57s and C57s transferred to DBAs showed increased alcohol consumption, findings which Randall and Lester conclude "uphold the hereditary basis of alcohol selection in C57BL mice, but show the avoidance of alcohol drinking by DBA mice to be amenable to environmental alteration".

Even if there were no questions about the findings themselves, there is no reason to regard an increase in consumption by one strain as less indicative of an environmental effect than the increase in consumption of the other. Both strains showed a similar effect.

It seems possible, however, that the composition of the *b* groups was not as represented. Randall and Lester state that experimental pups were identified by differences in eye pigment at birth. This method is liable to produce errors in identification, since both strains have pigmented eyes. Even if it was successful in the case of between strain (experimental) transfers it is difficult to see how it could have been used at all in the case of control transfers. The natural progeny of a C57 female mated to a C57 male will be indistinguishable from C57 transplants she has received (and, *mutatis mutandis*, the same problem arises with DBAs). It

seems likely, then, that groups 1b and 2b consisted of both control transplants and natural offspring. Given the small yield of transplants (three or fewer per litter), it is also likely that a majority of pups identified as control transplants were instead natural offspring, equivalent, except for the surgical stress to the mother, to the untreated controls.

Uncertainty about the composition of the control transplant groups precludes any conclusion about the effects of maternal environment: the increased alcohol consumption of the between-strain transplants could equally well be due to the transplant procedure in general or the surgical stress it entails. In this latter case, *b* groups (stressed) should have differed from *c* groups (unstressed), which they did not. The stress to the mother is, however, confounded with the transplantation procedure (affecting the ova?) in the *b* groups; if the two events oppose each other in their effects, the offspring might not differ from naturally bred ones.

Unfortunately, the confounding of variables does not permit any definite conclusion.

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¹ Randall, C. L., and Lester, D., *Nature*, 255, 147–148 (1975).

RANDALL AND LESTER REPLY—We agree with some of the arguments presented by Joffe, Najman and Nettleton¹, but we take issue with others. The difference in eye pigment between newborn C57BL and DBA pups is obvious at birth: C57BL pups have dark black eyes and DBA pups have lightly pigmented eyes. While differences in coat colour become evident within a few days and could be used, eye pigment allows immediate identification and the early and accurate culling of the litter.

In control transfers (C57BL–C57BL and DBA–DBA), the pups are, obviously, indistinguishable. We could have used mates of the opposite strain to ensure identification of the transferred ova, but this approach has its own inherent problems; we could also have mated females to infertile males of the same strain, but this was not a preferable method: the female is only pseudopregnant, no control uterine horn is provided and the transferred offspring are not intermixed with natural offspring. We thus considered other controls; without prior knowledge of the outcome, we chose the method described in our paper. We do not deny the validity of the arguments against this approach: the possibility certainly exists that natural, rather than experimental offspring, were examined. These other control procedures can, of course, also be

used to determine whether transplantation *per se* exerts no effect; should the results not agree, the issue would remain unresolved because of the differences in methods and the introduction of other confounding variables.

Preimplanted mouse embryos are resistant even to the most severe types of chemical and drug intervention, at least in regard to morphological development in a foster mother's uterus². Runner² suggests that nucleic information is not integrated until implantation occurs. Rather than attributing the increase in ethanol intake to the transplantation procedure *per se*—because surgery is performed at least 2 d before implantation—it is more likely that the outcome is a result of critical mother–foetal interactions. This issue is unresolved, however, since we do not know whether biochemical changes associated with implantation and pregnancy are similar in natural and transplanted offspring.

Maternal surgical stress does not explain the increase in alcohol intake of transferred young, unless we assume that transplanted ova are differentially sensitive to subtle changes in the maternal milieu or to subsequent postnatal events. In unpublished pilot work, we found anaesthesia alone did not alter phenotypic alcohol intake. Further, natural offspring tested concurrently with transferred pups did not demonstrate an atypical strain effect. For the sake of brevity and because we regarded the data as superfluous, these data were not in our report; obviously, in retrospect, they should have been included.

We can hardly argue that the alcohol consumption of C57BL and DBA mice is not subject to environmental alteration because we have produced such alterations³. The issue raised here is, however, the contribution of genetic and maternal variables to the strain extremes, since differences in intake are evident at weaning. We argue that if alcohol selection were determined by maternal rather than by genetic inputs, we should observe selections resembling the phenotypic norm of the foster mother. We noted no such effects in either strain: although alcohol choice increased in DBA mice transferred to C57BL mothers, the offspring never demonstrated C57BL-like high selection of alcohol.

Our report cannot be read to exclude the importance of environmental variables in determining behaviour, although it assuredly emphasises that maternal behaviour is not as important a determinant of alcohol intake as is genetic constitution.

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¹ Joffe, J. M., Najman, J., and Nettleton, N., *Nature*, 262, 725–726 (1976).

² Runner, M. N., in *Teratology: Principles and Techniques*, (edit. by Wilson, J. G., and Warkany, J.), (University of Chicago Press, Chicago, 1965).

³ Randall, C. L., and Lester, D., *Science*, 189, 149–151 (1975).

Molecular structure of NAD

VISWAMITRA¹ has speculated on the structure of free NAD which he then extrapolates to the NAD conformation when bound to enzymes. Unfortunately, he has neglected to take into account the extensive results which have been obtained during the past five years on the conformation of NAD when bound to lactate dehydrogenase², malate dehydrogenase³, liver alcohol dehydrogenase⁴ and glyceraldehyde-3-phosphate dehydrogenase⁵. Suffice it to say here that his speculations are inconsistent with facts and that the only 'fact' he does quote concerning the fit of his proposed NAD model to the low resolution difference map of Adams *et al.*⁶ is incorrect.

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¹ Viswamitra, M. A., *Nature*, 258, 540–542 (1975).

² Holbrook, J. J., Liljas, A., Steindel, S. J., and Rossmann, M. G., *The Enzymes*, XI (edit. by Boyer, P.), 191–292 (Academic, London, 1975).

³ Banaszak, L. J., and Bradshaw, R. A., *The Enzymes*, XI (edit. by Boyer, P.), 369–396 (Academic, London, 1975).

⁴ Brändén, C. I., Jörnvall, H., Eklund, H., and Furugren, B., *The Enzymes*, XI (edit. by Boyer, P.), 104–190 (Academic, London, 1975).

⁵ Buehner, M., Ford, G. C., Moras, D., Olsen, K. W., and Rossmann, M. G., *J. molec. Biol.*, 90, 25–49 (1975).

⁶ Adams, M. J., McPherson, A., Jr., Rossmann, M. G., Schevitz, R. W., and Wonacott, A. J., *J. molec. Biol.*, 51, 31–38 (1970).

VISWAMITRA REPLIES—The model proposed for NAD¹ was essentially for a free molecule. The reference to the work of Adams *et al.*² was to point out that NAD conformations with the adenine and nicotinamide bases far apart had been considered earlier. Although the statement about the possible compatibility of the model with the low resolution electron density maps of Adams *et al.* was unfortunate, it was only made as it was felt that electron density maps at 5-Å resolution, could admit, in general, a certain flexibility where the derivation of the shape of a small substrate molecule is concerned. It is also difficult to rule out conformational states of coenzyme molecules other than those derived from structural studies of bound enzymes as inconsistent with facts. The molecular structures found for ADP in the crystal structure of its rubidium³ and *tris* salts (M.A.V., Z. Shakked and O. Kennard, to be published) are different from those deduced for the coenzyme from structural studies of bound enzymes⁴.

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¹ Viswamitra, M. A., *Nature*, 258, 540–542 (1975).

² Adams, M. J., McPherson, A., Jr., Rossmann, M. G., Schevitz, R. N., and Wonacott, A. J., *J. molec. Biol.*, 51, 31–38 (1970).

³ Viswamitra, M. A., Hosur, M. V., Shakked, Z., and Kennard, O., *Nature*, 262, 234–236 (1976).

⁴ Chandrasekhar, K., McPherson, A., Jr., Adams, M. J., and Rossmann, M. G., *J. molec. Biol.*, 76, 503–517 (1973).

reviews

Genuine passion for research

E. H. S. Burhop

Frederic Joliot-Curie: A Biography. By Maurice Goldsmith. Pp.260. (Lawrence and Wishart: London, June 1976.) £6.

To the genuine scientific research worker there is no greater satisfaction than that of discovery. Frederic Joliot-Curie was just such a man with a genuine passion for research. In addition he was an unusually fine and talented craftsman, skilled with his hands and appreciative of beauty in technique no less than in art. He was married to Irene, daughter of Pierre and Marie Curie, born and bred to the laboratory and the exacting standards of scientific integrity and hard work, as well as the vision that characterised the work of her famous parents. There are three great highlights in the scientific work of the Joliot-Curies.

In 1931 they observed that a penetrating radiation emitted when beryllium was bombarded by α particles was capable of ejecting energetic protons from a thin layer of paraffin wax. They erroneously interpreted the penetrating radiation as due to 50 MeV nuclear γ rays but this was an unlikely explanation; and Chadwick, as soon as he heard of the work of the Joliot-Curies repeated it and established conclusively that the radiation consisted of neutrons, for which Rutherford had been searching for 13 years. For this Chadwick received the Nobel prize.

In 1934 they discovered the new phenomenon of artificial radioactivity, showing that light elements bombarded with α particles could be made radioactive, emitting positrons with characteristic half-life. For this discovery they shared a Nobel prize in 1935, just 32 years after a similar joint award to Irene's parents.

In 1939 Joliot and his colleagues studying the newly discovered process of nuclear fission after the capture of a neutron by uranium, showed that more than two neutrons were emitted in the subsequent fission, thus establishing the possibility of initiating a nuclear chain reaction and, incidentally, although they did not realise it at the time, of the breeder reactor.

Frederic and Irene Joliot-Curie could no doubt have derived the greatest satisfaction and sense of ful-



At a memorial meeting for Rutherford (November, 1947). Left to right: Desmond Bernal, Joliot-Curie, and Lady Rutherford

filment from continuing to work together in their laboratory for their whole lives. But they shared in their family backgrounds the finest traditions of French radicalism. They were conscious of the power of science and technology to provide the material basis on which a transformation of society could be effected. The reality they saw was of a society in which science was underused or misused, with poverty, unemployment, inequality, threatened internally and externally by fascism. War came and with it humiliation for their country.

Can a scientist remain tied to his laboratory, indifferent to what is going on about him in such circumstances? Or if he can, should he? The life of Frederic Joliot-Curie epitomises the problem of the social responsibility of the scientist. It is a tribute to Maurice Goldsmith that in this short and brilliantly written biography he not only captures the excitement and drama of the scientific discoveries to which the Joliot-Curies contributed in such ample measure. He also brings out the significance of the decisions Joliot had to

make and the deeper meaning of his life.

Joliot-Curie had, no doubt, what the answer to the above rhetorical question should be. He would have accepted the viewpoint of Paul Langevin, his teacher and friend: "I must devote myself to these political activities. It is true that I could be doing my science, but the science I could do will be done by others, and this task comes to me because it is not being done by others".

Joliot's sense of social concern led him along a hard and stony path, to great misfortune, and possibly to premature death for his wife and himself. He became Head of the World Peace Council. The great Stockholm Peace Appeal against nuclear weapons, signed by 500 million people throughout the world was his brainchild. This built up such a worldwide public reaction against nuclear weapons that it made the idea of a pre-emptive nuclear strike, for which some 'hawks' were clamouring, unthinkable. He became President of the World Federation of Scientific Workers which had been set up under the influence of scientists like Joliot,

Langevin and Bernal to try to ensure effective planning, policy making, peaceful application of science, and proper training and conditions of work for scientists. Joliot first raised with Bertrand Russell the possibility of the organisation of a world conference of scientists against nuclear weapons which eventually led to the Pugwash Conferences. The first contacts of the reviewer with both Joliot and Russell were connected with this initiative.

In the post-war period, alongside activities of this kind, Joliot was deeply involved in questions of French science and nuclear energy policy. Soon after the liberation of France he was given the task of reformulating science research policy. He reorganised the National Fund for Scientific Research (CNRS), laying a basis for French scientific research that persists today. On the setting up in October 1945 of the French Commissariat for Atomic Energy, Joliot was appointed its High Commissioner and in this capacity was responsible for planning the highly successful first decade of the French peaceful nuclear energy program.

But Joliot's increasing commitment to the struggle against nuclear weapons led to a virulent campaign against him

in the French press and increasing pressure on the French Government to dismiss him. Eventually in 1950 his one time friend and colleague in the Resistance, Prime Minister Georges Bidault, delivered the *coup de grace*.

Joliot was a sensitive man and his dismissal and the abusive and deeply wounding insinuations that accompanied it saddened his last years. Certainly Irene, to whom he was devoted, and possibly he himself, suffered from the effects of radiation. He died in 1958, surviving Irene for two years.

This is a significant book and successfully portrays the warmth, greatness and vision of its subject. Some parts read as excitingly as a detective novel, as when it describes how Joliot's foresight and initiative led to his arranging for France to acquire the whole Norwegian stock (~ 180 litres) of heavy water to prevent its falling into German hands; and then arranging for its transfer to Britain, just ahead of the Nazi invasion of France. This was a signal contribution to the Allied cause for which he received scant recognition when he was refused permission to enter Britain in 1950 to attend the Sheffield Congress of the Partisans of Peace. Again there is the account of

his courageous work in the French resistance, especially in the preparations for the Paris rising of 1944, for which his laboratory organised the supply of munitions.

But the key question that will be raised by the book and one that still confronts us today is the way in which the scientist must reconcile his duty to his profession of scientific research worker with his responsibility to society as a whole. Joliot had to make this decision under circumstances of very great difficulty and there will certainly be differences of view concerning the correctness of his choice. If, however, the 500 million signatures of the Stockholm Peace Appeal helped significantly to prevent the further use of nuclear weapons then surely one must conclude Joliot's choice was the correct one. □

Professor E. H. S. Burhop, FRS, is Professor of Physics at University College, London, UK. He was associated with F. Joliot-Curie in the World Federation of Scientific Workers and eventually succeeded him and C. F. Powell as President.

Marine mussels

Marine Mussels: Their Ecology and Physiology. (International Biological Programme 10.) Edited by B. L. Bayne. Pp. xviii + 506. (Cambridge University: Cambridge and London, June 1976.) £22.

THIS excellent volume is strongly recommended for purchase by universities, polytechnics, museums, and marine biological research stations, on a worldwide basis. It will be an indispensable tool for professional biologists who are concerned with biology of bivalve molluscs, or productivity of coastal waters, and who can afford the price. It will form a stimulating basis for university courses in marine biology.

The editor and authors are to be commended for their lucid, narrative style, for every chapter makes interesting reading. The authors' authority lies in their own contributions to the literature. They comprise: Sir Maurice Yonge (The 'mussel' form and habit); R. Seed (Ecology); D. Roberts (Mussels and pollution); B. L. Bayne (The biology of mussel larvae); B. L. Bayne, R. J. Thompson and J. Widdows (Physiology I, II, Physiological integrations); P. A. Gabbott (Energy metabolism); J. S. Levinton and R. K. Koehn

(Population genetics of mussels); J. Mason (Cultivation).

Mussels have a global distribution in coastal waters and estuaries, and provide a major part of the world crop of bivalve shellfish; and they are important components of their ecosystems. The book deals with the whole range of biology of mussels—how their physiological attributes equip them for their environments and the variables with which they contend. We are informed on the biology of mussel larvae, their food preferences, primary settlement, and subsequent movement to beds of adult mussels. Growth rates at different tidal levels, predators, and parasites, are considered in turn.

Physiology of nutrition, and respiration, and the functioning of the vascular and excretory systems are studied in depth. Physiological features are related to the ecological setting—for example, the ability of mussels to acclimate their filtration rate and oxygen consumption (but not heart beat) to short-term fluctuations in temperature and ration.

Seasonal changes in biological composition of mussel flesh, food reserves, metabolic pathways, and control mechanisms are considered, the last with reference to compensation for environmental change. Population genetics, with reference to isoenzymes, reveals variation on a geographic scale,

and also on a small scale within an estuary or tidal flat.

Consideration is given to pollutants, and to purification before marketing; care must be taken against paralytic shellfish poisoning arising from unusual blooms of *Gonyaulax* or *Gymnodinium*. The book ends with a review of mussel cultivation in Western European seas.

The book is illustrated by 142 text figures which are commendably clear; most of these are graphs and line diagrams, but there are also some good photographs. The reference list, which must contain about 1,500 entries, will be a valuable tool in itself. There is a good index.

Typographical errors are rare. The Reviewer regrets the general misuse of the word 'visualisation' by chromatographers, and the increasing mistreatment of 'data' as a singular noun; and he is disappointed to find both these usages here. What, incidentally, is 'vagile' (p 358)—it is not in the reviewer's copy of the Shorter Oxford English Dictionary. **R. D. Purchon**

R. D. Purchon is Professor of Zoology in the University of London, Head of the Department of Zoology at Chelsea College, and presently the Chairman of the Biological Sciences Group of Departments at Chelsea College, London, UK.

Primitive sensory systems

Primitive Sensory and Communication Systems: The Taxes and Tropisms of Micro-Organisms and Cells. Edited by M. J. Carlile. Pp. vii+258. (Academic: London, New York and San Francisco, January 1976.) £8.50; \$21.

THERE has been a renewed interest and considerable progress in the study of cell orientation, and this compact book shows some of the new results and the new excitement. If I had any criticism, it would be one of omission rather than what the book contains.

It starts off with a clear and systematic introductory essay by M. J. Carlile. The effects of light on orientation are treated in an organised fashion by W. Nultsch. Julius Adler provides a short, lucid review of his own beautiful work; I am only sorry he did not go into further detail, especially on the work of Berg, Koshland and others; but there are a number of other good recent reviews on this subject published elsewhere. T. M. Konijn provides the best review I have seen anywhere of all the work

on cellular slime mold chemotaxis, and I feel equally enthusiastic about G. W. Gooday's review of chemotaxis (and tropisms) in the algae and fungi.

Finally, P. C. Wilkinson provides a brief review of the work on leukocyte chemotaxis which is succinct and clear, as one would have expected if one has read his fine book on the same subject. He stresses the point that macromolecules seem to be important leukocyte attractants, which contrasts with the role of small molecules as attractants in the earlier chapters.

Besides more on bacterial chemotaxis, I would have liked to see a review of all the work on pollen tubes; it would have fitted in well. I also missed a concluding chapter which might have attempted to bring together some of the photo- and chemo-orientation ideas, as well as some of the common and contrasting details in the different chapters. But perhaps these complaints are merely an indication that I found myself stimulated (and oriented!) by the book.

J. T. Bonner

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Trace elements

Trace Element Analysis. By V. Valkovic. Pp. x+229. (Taylor and Francis: London, November 1975.) £7.

Trace Element Analysis is a unique book as far as its content can be judged in relation to its title. There are many books which treat techniques of trace analysis in monographic form or are devoted to a wide range of physicochemical techniques but which in general say little about the reasons or rationale for doing such work. This slender attractive volume does set out to describe some techniques, but concentrates to an unusual degree on setting the scene and giving the background to the philosophy and fundamental reasons for the importance of analysing for trace elements under particular sets of circumstances. This part of the book is extremely well done and the author is to be congratulated on his grasp of such a wide field of knowledge and his ability to write in such an interesting way. This part of the text (128 pages) occupies the first six chapters: elements in nature; trace elements in the environment; environmental pollution; trace elements in biology and medicine; general aspects of trace analysis.

The remaining 67 pages are devoted to chapters on activation analysis, X-ray emission spectroscopy, optical methods and mass spectrometry. Here the book is not quite so satisfactory in that the treatment is more restricted and a good deal less detailed and rather less critical in its assessment than other books which are available. It is, for example, disappointing to find no mention of work on electroanalytical techniques of trace analysis and scant attention given to the preconcentration techniques that are so important to trace analysis in all its guises. Some of the descriptions of atomic spectroscopic techniques are also rather outdated and oversimplified.

The strength and the excellence of this book lies, however, in the first six chapters which are unusually good and make it a worthwhile text for all who are concerned with the broader aspects of trace element analysis.

T. S. West

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Radiation and matter

The Dynamics of Spectroscopic Transitions: Illustrated by Magnetic Resonance and Laser Effects. (Wiley-Intersciences Monographs in Chemical Physics.) By James D. Macomber. Pp. xxiv+332. (Wiley-Interscience: New York and London, February 1976.) \$23; £11.50.

THIS book is concerned with the mechanisms by which radiation can interact with matter and, in particular, with the processes involved when electromagnetic radiation stimulates a transition from one quantised energy level to another. The author points out that discussions of the physical principles involved are often omitted in books on spectroscopy and has directed this book at advanced undergraduates, graduates and research spectroscopists. The first three chapters outline the essential quantum theory and theory of electromagnetic radiation that is required. Chapters 4 and 5 give the theory of the interaction of radiation with matter, and in chapter 6 the theory is illustrated with reference to magnetic resonance; in this area the

physical principles are particularly easily understood. In the last two chapters the theory is generalised to other systems and to two-level systems.

The subject inevitably requires mathematical treatment, and this is set out very clearly in the book. At the same time the author is at pains to give, wherever possible, physical explanations of the processes involved. These are often of great value in thinking about spectroscopic experiments, and many spectroscopists would find much to fascinate and enlighten them.

I was a little surprised to find in the historical introduction no mention of Purcell, Torrey and Pound as discoverers of magnetic resonance in bulk matter at the same time as Bloch and his colleagues. There are one or two minor and obvious errors (for example in Fig. 4.3), but these do not significantly mar a clear and lucid account of an important aspect of spectroscopy.

R. E. Richards

Dr Richards is Warden of Merton College, Oxford, and Chairman of the Oxford Enzyme Group working in the Department of Biochemistry at the University of Oxford, UK.

obituary

Georgii Samsonovich Dzotsenidze, the eminent Soviet geologist died on May 5, 1976, aged 66.

Dzotsenidze was born in 1910, in the Georgian town of Kutaisi. He was educated at Tbilisi University, graduating in 1929, and from then on his academic career was closely connected with the University, rising from the position of laboratory assistant in the Department of Mineralogy and Petrology, to become a lecturer, Dean of the Faculty of Geology and Geography, and finally, in 1958, Rector of the University. His research was concerned mainly with the geology of Georgia; in 1933–34 he led the geological survey

team of the Georgian Geological Board. His more than 120 publications include *Premiocene extrusive vulcanism of Georgia*, *Question of the genesis of barytic deposits in Georgia*, *Analcite level in the coal-bearing formation of the Kutaisi–Gelati region*, and *Petrology of the Bath Deposits of Okribri*. He was elected a full member of the Georgian Academy of Sciences, having served for four years as acting Academic Secretary of the Academy. In the same year (1955), he became a Vice-President of the Georgian Academy of Sciences. In 1968 he became a full member of the Academy of Sciences of the USSR.

Dzotsenidze combined his academic career with considerable political activity. He served as a Deputy of the Supreme Soviets of the Georgian SSR and the USSR several times, and was a member of the Central Revision Commission of the CPSU and of the Central Committee of the Communist Party of Georgia.

For his academic and political activities Dzotsenidze received a number of high Soviet honours, including the Order of Lenin (three times), the order of the October Revolution, the Order of the Red Banner of Labour, a Stalin (now State) prize, and numerous medals.

announcements

The **1976/77 Moscow Seminars on Collective Phenomena** will take place on Sundays at 1200 starting on September 12, at Ulitsa Veshnyakovskaya, Dom 4, Korpus 2, Apartment 5, Moscow. Letters, reprints and preprints may be sent to **Professor M. Ya. Azbel'** at the above address, but all correspondence must be registered and insured. All visiting scientists are very welcome to attend the seminars unannounced.

The programme is:

September 12, 1976: Dr Yu. Mniukh on **Polymorphic Transitions in Crystals**.

September 19, 1976: Professor Yu. Orlov on **Wave-logic and its Applications to Wave-Mechanics**.

September 26, 1976: Dr A. May on **Cybernetic Ideas and Empirical Sociology**.

October 3, 1976: Corresponding Member B. Levich on **The Semi-empirical Theory of Turbulence**.

October 10, 1976: Professor M. Ya. Azbel' on **Decoding DNA**.

October 17, 1976: Dr I. Andryukin on **The Theory of Exactness of Mechanical Treatment**.

October 24, 1976: Dr E. Trifonov on **Genetic Engineering**.

October 31, 1976: Dr G. Rosenstein on **Models of Mental Distress**.

November 7, 1976: Dr V. Brailovsky, on **Deducing Relationships from Experimental Data**.

November 14, 1976: Professor N. Salanski on **Physics of Magnetic Films**.

November 21, 1976: Dr Yu. Kalenov on **Hydrodynamic Lasers**.

November 28, 1976: Dr V. Lazaris, on **The Creativity of Ben Galevi**.

December 5, 1976: Professor Ya. Alpert on **Wave-Phenomena in Cosmic Plasmas**.

December 12, 1976: Dr A. Kaplan on **Dynamics of Quantum Systems in a Non-linear Resonance Field**.

December 19, 1976: Professor N. Meyman on **Spectral Properties of the Schrödinger Equation in a Periodic Field**.

December 26, 1976: Professor B. Fain on **An Experimental Investigation of Psychological Peculiarities**.

January 2, 1977: Dr I. Brailovskaya on **Supersonic Viscous Flow Around a Body**.

January 9, 1977: Dr I. Gildengorn on **The Influence of Oxidation on Thermoelectric Properties of Alloys**.

January 16, 1977: Dr M. Shepelev and Dr V. Dubrovskaya on **The Order of Reaction in Vulcanisation of Rubber**.

January 23, 1977: Professor A. Tzinobor on **Hydrodynamics of Liquid Metals in a Magnetic Field**.

January 30, 1977: Professor N. Salansky on **Yoga and Judaism**.

February 6, 1977: Corresponding Member B. Levich on **Electrocapillary Phenomena**.

February 13, 1977: Professor M. Ya. Azbel' on **Entropy and Phase Transitions**.

February 20, 1977: Dr A. Schuster on **An Optical Problem in Combination Theory**.

February 27, 1977: Dr G. Shapiro on **Hormonal Regulation of Vital Functions**.

March 6, 1977: Dr L. Regelson on **Interaction of Weak Fields with Biological Structures**.

March 13, 1977: Professor N. Meyman

on **Dispersion Relations and the Flow Increase Principle**.

March 20, 1977: Dr V. Relz on **Determination of the Electronic Density Function of Protein**.

March 27, 1977: Dr L. Vilenskaya on **Possible Interpretation of some Psychological Phenomena**.

April 3, 1977: Professor M. Kovner on **Earth's Radiative Belts and their Origin**.

April 10, 1977: Dr A. Kaplan on **Non-linear Fresnel Reflection**.

April 17, 1977: Professor S. Alber on **Asymptotic Methods in the Theory of Eigenvalues**.

April 24, 1977: Dr E. Tzifonev on **Evaluation of Nucleotide Sequences (Experimental)**.

May 1, 1977: Professor B. Fain on **Non-radiative Transitions in Condensed Matter**.

May 8, 1977: Corresponding Member B. Levich on **Surface Turbulence in a Liquid**.

May 15, 1977: Dr G. Volk on **Viscosimetric Determination of Molecular Weights**.

May 22, 1977: Dr A. Dolgopolsky on **The Hebrew Language and Alphabet: The History of Languages**.

May 29, 1977: Professor M. Ya. Azbel' on **New Statistics of Quasiparticles**.

June 5, 1977: Dr A. Malitzky on **The Planning of an Experiment**.

June 12, 1977: Dr E. Tzifonev on **The Spatial Structure of RNA**.

To get to Professor Azbel's apartment: Take the Metro to Zhdanovskaya (end of line in Eastern suburbs), then the No 64 Trolleybus North along Ulitsa Veshnyakovskaya until the road forks (about 6 stops).